HEREDITY VOLUME 16 PART 2 MAY 1961

# FITNESS AND ARTIFICIAL SELECTION

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Received 29.xii.60

## 1. INTRODUCTION

It is usual to find that viability and fertility decline in lines that are subjected to selection for metric characters not evidently directly concerned with these components of fitness. When the artificial selection is relaxed, natural selection tends to restore viability or fertility, and there is corresponding loss of some of the results of selection (see Lerner, 1954).

Mather and Harrison (1949) have argued that such results are in no simple way to be interpreted in terms of pleiotropic effects, their evidence pointing to linkage of fertility genes to those affecting the primary character under selection. Breese and Mather (1960) have obtained confirmatory evidence, and the results of Clayton, Morris and Robertson (1957) accord with this view in showing that the rate and extent of reversion under relaxed selection are not completely correlated with the extent of response under forward selection.

The extent to which the response to selection for a character, such as bristle number in *Drosophila*, may be affected by fertility genes not directly influencing that character itself, could be conveniently tested if it were possible to assess the effect on a selection line of a gene with no detectable effect on bristle number, but with considerable effect on viability or fertility. The occurrence of a mutation of this kind in a line of *Drosophila melanogaster* under selection for sternopleural chaeta number gave us the opportunity to perform such a test. The results are reported here.

## 2. MATERIALS AND METHODS

### (i) *Material*

The mutant was found as a single male in the fifteenth generation of a line under selection for low chaeta number. The selection line was homozygous *vg* having been initiated from the vestigial segregants in the $F_2$ of a cross between a vestigial stock and inbred Oregon wild type. The mutant male had strap-like wings. It was mated to a single vestigial sister and produced 58 strap and 40 vestigial progeny, a ratio not significantly different from 1 : 1 ($\chi^2 = 3{\cdot}31$) but having a suggestive excess of "strap". "Strap" flies from this progeny were mated together and their progeny contained 151 wild type, 356 "strap", and 122 vestigial flies, a significant deficiency of vestigial. These results suggested that the mutant arose from a partial back mutation of *vg* to an allele similar to the pennant alleles (Bridges and Brehme, 1944) and that the mutant allele raised the viability of flies relative to *vg*.

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The mutant might of course have been a suppressor at another locus, but this possibility was virtually eliminated. Homozygous " pennant " flies were mated to wild type and $F_1$ females were mated to *vg*/*vg* males. Some 13,000 progeny were obtained and these were wild type and heterozygous pennant/vestigial (" strap ") in 1 : 1 ratio. No vestigial progeny occurred. The mutant was therefore designated $vg^{pD}$ (vestigial pennant of Dinsley).

One pair of offspring from the original mutant male and his mate (vestigial sister) was used to start a $vg^{pD}/vg$ stock which was maintained thereafter by transferring five pairs of heterozygotes each generation. Three generations later this stock was used to establish two low selection lines, one homozygous pennant and the other homozygous vestigial.

In addition, two backcross lines were started in order to transfer the pennant gene into a high vestigial line (" vg 6 " of Thoday and Boam, 1961) that was thought to be approaching a plateau. It was not near its plateau, but continued responding for a further 40 generations, so the two backcross lines were maintained for this time. This was done by crossing $vg/vg^{pD}$ females to males of " vg 6 " in each generation, and repeating with the heterozygous female progeny in each subsequent generation. Selection lines were in due course taken from these backcross lines as will be described below.

### (ii) *Selection Methods*

The selection lines to be described here were maintained exactly as other directional selection lines maintained in Sheffield (see Thoday, 1958). There were four single-pair cultures per generation, with a rotational mating system maintaining the four female sub-lines as one population. Twenty of each sex from each culture of each generation were assayed for chaeta number, and the best fly from each 20 was used to maintain the line. The next three flies were set up in three further single-pair cultures to insure against failure of the first culture, so that 16 single pairs were set up per line per generation. The number of flies emerging in the first four days of emergence in the first successful culture of each sub-line was recorded as a measure of fertility and viability and is referred to below as the productivity of the line. The number of single-pair cultures that proved sterile was also recorded.

## 3. RESULTS

### (i) *Vestigial, pennant and fitness*

Tests were made to confirm the conclusion, suggested by the initial data, that the pennant gene increased the fitness of flies relative to vestigial.

One test was made by determining the effect of natural selection on heterozygous populations. Six population bottles were set up from the $vg^{pD}/vg$ stock. By the tenth generation the gene *vg* had been eliminated from all the six populations. No homozygous *vg* flies occurred after the sixth generation. In these conditions natural selection strongly favoured pennant at the expense of vestigial. Part of this result was shown to arise from the greater success of pennant males in obtaining mates, the inferiority of vestigial males being pronounced. Pennant homozygotes were somewhat superior to heterozygotes in this respect.

The first generation of these populations, being progeny of pennant heterozygotes, provided a test of relative viability. There were 207 homozygous pennant, 337 heterozygotes, and 127 homozygous vestigial flies, a clear deviation from the 1:2:1 ratio to be expected if there were

no viability disturbances ($p<0{\cdot}001$). These data are consistent with expectation based on $(p+q)^2$, and thus provide no evidence for heterosis ($p<0{\cdot}7>0{\cdot}5$). Six further comparable tests were made and gave a total (excluding those above) of 760 pennant, 1327 heterozygous and 568 vestigial flies, against an expected 663·75, 1327·5, 663·75, and leave no doubt of the effect of pennant on viability during larval and/or pupal development.

Tests were also made to determine whether pennant and vestigial flies differed in fertility. Table 1 shows the number of progeny produced by 18 successful single-pair cultures of each of the four types of cross. The parents came from the *vg*$^{pD}$/*vg* stock. Thirty-six cultures had been set up and the number of sterile matings is recorded. There

TABLE 1

*Fertility of vestigial and pennant flies*

| Cross female × male | Sterile matings | No. of progeny from 18 fertile cultures |
|---|---|---|
| *vg*$^{pD}$/*vg*$^{pD}$ × *vg*$^{pD}$/*vg*$^{pD}$ | 0 | 2172 |
| *vg*$^{pD}$/*vg*$^{pD}$ × *vg*/*vg* | 11 | 2250 |
| *vg*/*vg* × *vg*$^{pD}$/*vg*$^{pD}$ | 3 | 1556 |
| *vg*/*vg* × *vg*/*vg* | 18 | 1313 |

is no doubt that matings involving vestigial males are more often sterile ($p<0{\cdot}001$), and there is similar evidence, though less strong, that the same applies to vestigial females ($p<0{\cdot}05>0{\cdot}02$). It seems further that, if successfully mated, pennant females produce more progeny than vestigial females.

The origin of pennant, and the method of establishment of the *vg*$^{pD}$/*vg* stock, make it rather unlikely that these effects are due to genes at any locus other than *vg*. We conclude that *vg*$^{pD}$ is a gene that has a considerable effect on many aspects of fitness, making its possessors superior to *vg*/*vg* flies.

### (ii) *Pennant, vestigial and chaeta number*

The mean sternopleural chaeta numbers of the pennant, heterozygous and vestigial flies in 6 *vg*$^{pD}$/*vg* sub-lines of the *vg*$^{pD}$/*vg* stock are listed in table 2. Each entry is based on 20 flies of each sex from each of four cultures. The analysis of the variance of these means shows that there is some evidence that the different sub-lines differ in chaeta number, but there is no evidence that the genes *vg*$^{pD}$ and *vg* have any differential effect on chaeta number.

### (iii) *Low selection lines*

As described on p. 114, two selection lines were established from the *vg*$^{pD}$/*vg* stock, one homozygous pennant and the other homozygous vestigial. These lines were maintained for 25 generations.

Figure 1 illustrates the results of selection. For the first 12 generations the two lines responded together, and there is no evidence that their chaeta numbers differed. The productivity of the pennant line was, however, much higher than that of the vestigial line. After the 12th generation response of the vestigial line ceased, but response of the pennant line continued. There was a coincident fall in the productivity of the pennant line, which brought its productivity down to the level of that of the vestigial line.

TABLE 2

*Mean sternopleural chaeta numbers of segregants from 6 $vg^{pD}/vg$ stocks*

| Stock | Genotype of offspring | | |
|---|---|---|---|
| | $vg^{pD}/vg^{pD}$ | $vg^{pD}/vg$ | $vg/vg$ |
| 1 | 15·77 | 15·82 | 16·07 |
| 2 | 16·24 | 16·12 | 16·10 |
| 3 | 16·01 | 15·86 | 16·00 |
| 4 | 15·69 | 15·71 | 15·95 |
| 5 | 15·94 | 15·81 | 15·87 |
| 6 | 15·85 | 15·92 | 15·90 |
| Mean 1-6 | 15·92 | 15·87 | 15·98 |

*Analysis of variance*

| Source | n | Mean square | P |
|---|---|---|---|
| Genotypes . | 2 | 0·01785 | 0.2 |
| Stocks . . | 5 | 0·04714 | <0·05>0·01 |
| Interaction (error) | 10 | 0·00944 | ... |
| Total . | 17 | ... | ... |

It might be supposed that the vestigial line was, by generation 11, homozygous at all loci affecting chaeta number, but this was not so. The mean chaeta number of a relaxed selection line taken from it at that generation rose significantly.

Not only were the productivities of the two lines different in the first 10 generations, but their " sterilities " also differed. In the first 10 generations 10 single pair matings failed in the pennant lines (16 set up each generation (see p. 114)). There were 70 comparable failures in the vestigial line. In the second 10 generations 26 failed in the pennant and 64 in the vestigial lines. In 10 generations of relaxed selection there were 13 pennant failures and 40 vestigial failures.

We therefore have a strong suggestion that the greater " fitness "

of pennant does permit more extreme results from selection. At the same time, there is no evidence that it affects the initial rate of response to selection.

### (iv) *The first high selection lines*

As vg 6 had been expected to reach a plateau soon after the backcrossing programme was begun, high pennant and vestigial selection lines were set up after only 7 generations of backcrossing. Three pairs of sister selection lines, each comprising one *vg*/*vg* and one $vg^{pD}/vg^{pD}$

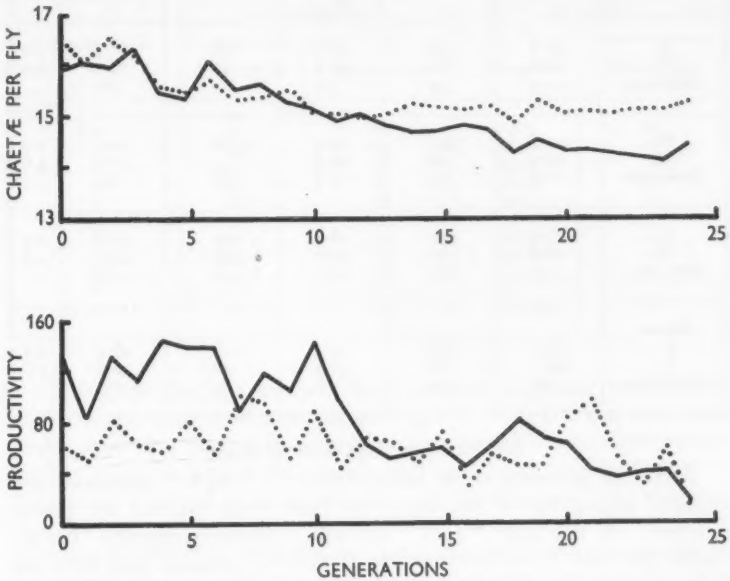


FIG. 1.—The mean chaeta numbers and the productivities of the pennant and vestigial low selection lines. Solid lines : pennant. Dotted lines : vestigial.

line were started from three separate cultures of one of the backcross lines.

As might be expected, the chaeta numbers of the *vg*/*vg* lines were at the start higher than those of the pennant lines. Productivity of the pennant lines was higher than that of the vestigial lines. The lines were selected for high chaeta number for 11 generations, after which other commitments forced us to cease selection. They were maintained thereafter by 6-pair transfers without selection for a further 20 generations. The results are summarised in table 3.

The table shows clearly the greater productivity of the pennant lines. There is evidence of a decline of productivity with selection in the vestigial lines, but negligible evidence of a similar decline in the pennant lines. There is no evidence that this productivity difference

affected the rate of response to selection. There is, however, strong evidence that it did affect the changes under relaxed selection, for the pennant lines lost fewer chaetæ than the vestigial lines.

TABLE 3

*Results from the first high lines*

| Lines | Initial chaeta no. | Initial productivity | Chaeta no. after selection | Productivity after selection | Chaeta no. after relaxing selection | Fall |
|---|---|---|---|---|---|---|
| $P_1$ | 27·7 | 115 | 31·2 | 110 | 30·2 | 1·0 |
| $V_1$ | 29·2 | 82 | 31·1 | 70 | 23·1 | 8·0 |
| Difference | −1·5 | 33 | 0·1 | 40 | 7·1 | ... |
| $P_2$ | 27·6 | 100 | 29·4 | 80 | 27·7 | 1·7 |
| $V_2$ | 29·5 | 76 | 32·3 | 44 | 27·7 | 4·6 |
| Difference | −1·9 | 24 | −2·9 | 36 | 0·0 | ... |
| $P_3$ | 27·2 | 130 | 28·4 | 125 | 27·1 | 1·3 |
| $V_3$ | 28·8 | 70 | 30·5 | 35 | 29·0 | 1·5 |
| Difference | −1·6 | 60 | −2·1 | 90 | −1·9 | ... |
| Means | | | | | | |
| P | 27·5 | 118 | 29·7 | 105 | 28·4 | 1·3 |
| V | 29·1 | 76 | 31·3 | 50 | 26·6 | 4·7 |
| Difference | −1·6 | 42 | −1·6 | 55 | 1·7 | ... |

### (v) *The second high selection lines*

After 22 generations of backcrossing to "vg 6", pennant and vestigial segregants of the backcross lines were assayed for chaeta number. At this time "vg 6" was still responding rapidly. Nevertheless the tests demonstrate (table 4) that the pennant flies from the

TABLE 4

*Assays of the backcross lines after 22 generations backcrossing to vg 6*

| | Backcross line | Class of fly | | |
|---|---|---|---|---|
| | | Pennant | Heterozygous | Vestigial |
| Chaetæ/fly . | 1 | 40·7 | 40·2 | 42·6 |
| | 2 | 40·8 | 40·2 | 41·8 |
| Total flies . | 1 | 147 | 296 | 48 |
| | 2 | 86 | 186 | 45 |

backcross lines were closely approaching the very high chaeta numbers of the vestigial segregants. They also provide evidence that the viability of pennant flies was still greater than that of vestigial flies.

After 40 generations of backcrossing, the line " vg 6 " had reached a peak of 46 chaetæ, and fallen back somewhat. At this stage a new homozygous pennant high selection line was extracted from one of the backcross lines and run parallel with " vg 6 " itself. The results are given in table 5.

TABLE 5

*Mean sternopleural chaeta number and productivity in the $vg^{pD}/vg^{pD}$ high line and in the vg 6 (vg/vg) high line during the same period*

| Generations | $vg^{pD}/vg^{pD}$ | | *vg6 (vg/vg)* | |
|---|---|---|---|---|
| | Chaetæ/fly | Productivity | Chaetæ/fly | Productivity |
| S o | 42·0 | 33 | 43·3 | 28 |
| 1 | 43·8 | 47 | 43·4 | 26 |
| 2 | 45·2 | 50 | 43·9 | 19 |
| 3 | 45·8 | 50 | 41·9 | 24 |
| 4 | 44·8 | 13 | 44·5 | 25 |
| 5 | 44·6 | 23 | 43·2 | 20 |
| 6 | 46·3 | 44 | 45·2 | 25 |
| 7 | 45·1 | 20 | 44·8 | 23 |
| 8 | 45·6 | 21 | 44·9 | 20 |
| Mean 1-8 | 45·150 | 33·50 | 43·975 | 22·75 |

It is clear that the pennant line preserved a greater productivity and a higher chaeta number than " vg 6 ". " vg 6 " was also more sterile, and after the two lines had been run together for 11 generations further selection became impossible as " vg 6 " had become too sterile to maintain except in mass culture. Once again we have evidence that the gene $vg^{pD}$ increases the productivity of a selection line, relative to vestigial, and that, as in the low lines, this permits the maintenance or achievement of more extreme chaeta numbers. Admittedly these two lines are less strictly comparable than the low selection pair, as the high vestigial line with which the pennant line is compared was not itself segregated from the $vg^{pD}/vg$ backcross line flies. Other commitments at the time prevented us maintaining the further selection line this would have involved. It seems unlikely, however, that this can seriously affect the validity of the comparison.

## 4. DISCUSSION

The results described above demonstrate clearly that the mutant $vg^{pD}$, which arose in a *vg/vg* low selection line had a considerable effect on the fitness of flies possessing it. All the tests, and all the selection lines in which $vg^{pD}$ was later used, agree in showing that $vg^{pD}$ increased the productivity of flies relative to that of flies of closely similar genetic background but homozygous for the gene *vg*, and the differences in frequency of sterile cultures are striking. There is also

good evidence, both from the specific tests and from the pair of low lines in their earlier history, that the genes *vg* and $vg^{pD}$ have negligible differential effect on sternopleural chaeta number.

Both pairs of selection lines, the low lines and the second high lines, provide evidence that the gene $vg^{pD}$ permits selection to push chaeta numbers to more extreme values than does the gene *vg*. It seems fair to conclude that it is the greater fertility produced by $vg^{pD}$ that makes this possible. This is to conclude that the contribution of $vg^{pD}$ to fitness has an additive component which to some extent compensates for the effects of other genes, or gene complexes, selected as a result of selection for extreme chaeta number. Hence the pressure of natural selection against these other genes is reduced in the presence of $vg^{pD}$, and artificial selection can therefore exploit the chaeta number genes more fully.

The behaviour of the first high lines under relaxed selection is in agreement with this conclusion. In the presence of $vg^{pD}$, natural selection pressure was less effective in reducing chaeta number in these lines when the opposing artificial selection pressure was removed.

These results, therefore, support the views and the evidence of those workers who have supposed that correlated responses in " fertility " are responsible for the barriers that are so often met in selection experiments even when the existence of genetic variance of the character under selection can be demonstrated. They further support the conclusions of Mather and Harrison (1949) concerning the possibility that linked fertility genes without pleiotropic effects on chaeta number may often be involved. They seem to demonstrate that the depressed fertility consequent on selection for chaeta number, high or low, whether itself due to pleiotropy or linkage, can be in part compensated for by other loci that have no detectable direct influence on chaeta number. Chaeta number and fertility in a selection line at a plateau are therefore at least partly separable, as Mather and Harrison's experiment also demonstrated.

## 5. SUMMARY

1. In a homozygous *vg*/*vg* line that was under selection for low chaeta number, a mutant fly occurred with strap-like wings. The mutant fly was shown to be heterozygous for *vg* and a gene similar to the " pennant " alleles of *vg*.

2. The mutant gene greatly increased the fitness (viability, fertility and mating success) of its possessors relative to *vg*/*vg* flies, but it had no detectable effect on chaeta number.

3. Sister low chaeta number selection lines were set up from the progeny of the mutant fly and its mate. For 11 generations they had similar though decreasing chaeta numbers, but the pennant line had much greater productivity (number of flies produced per culture). Thereafter response to selection by the *vg*/*vg* line ceased, but the

pennant line continued to respond. At the same time the productivity of the pennant line fell to that of the *vg/vg* line.

4. The pennant gene was also backcrossed for 40 generations to a high chaeta number *vg/vg* selection line. A homozygous pennant high line was then set up and run parallel with the high *vg/vg* line under selection for high chaeta number. The pennant line had higher productivity and maintained higher chaeta numbers.

5. These and other lines showed that the introduction of a gene (pennant) that increased " fitness ", but did not of itself affect chaeta number, to a line reaching the limits of selection for chaeta number, could permit that line to maintain more extreme chaeta numbers. The " fitness " gene also reduced the rate of regression under relaxed selection. It did not, however, affect the rate of forward response until the selection lines approached their plateaux.

6. It is concluded that chaeta number and the fitness of a line at its selection plateau are at least in part affected by different genes.

*Acknowledgments.*—We would like to acknowledge the help of Mr T. B. Boam, and a grant for technical assistance from the Agricultural Research Council.

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# VARIATION IN THE MINERAL COMPOSITION OF RYE SEEDLINGS

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Received 30.xi.60

## 1. INTRODUCTION

THE improvement of crop quality by the plant breeder is achieved directly or indirectly by selection for heritable chemical differences in the composition of individual phenotypes. Improvement of yield must also inevitably result in the selection of chemical differences either as causes or consequences of vigorous plant development. This being so it is perhaps surprising that comparatively little attention has been devoted, in higher plants at any rate, to investigating directly the variation in chemical composition of plants within and between populations (see Wagner and Mitchell, 1955).

The present work was the beginning of an inquiry into the variation in plant mineral composition occurring *within* a population. For this purpose a comparison was made between three rye families each derived by inbreeding from the same initial population—the variety Stålråg. The advantage of using inbred families is that potential variation is released when inbreeding is imposed on normally outbreeding populations such as occur in rye. This greatly facilitates the detection of the inherent variability of the original population. In this work the minerals investigated were potassium, phosphorus and iron.

## 2. MATERIAL AND METHOD

As mentioned earlier the three families used were all derived from the Swedish variety Stålråg. Two were homozygous lines, *P6* and *P17*, inbred by self-pollination for more than thirty generations. The third family was an $F_2$ produced by self-pollinating an $F_1$ hybrid between *P6* and another line.

Seedlings were raised in soil (J.I. Potting Compost) in boxes. Five weeks after sowing the shoots of each plant were cut off complete and analysed separately for the three mineral components. At this stage all plants were at a corresponding stage of development, each having developed three vegetative tillers. The methods for mineral analysis were as follows :—

1. Plants were dry-ashed in a muffle furnace, according to the method of Peech (see Jackson, 1958).
2. The plant extract was taken up in 2 per cent. hydrochloric acid.
3. Potassium was estimated on an Eel flame photometer.
4. Phosphorus determination was by the stannous chloride-reduced molybdenum blue method (Jackson, 1958).
5. Iron was estimated using Bathophenanthroline (Smith, McCurdy and Diehl, 1952).

## 3. RESULTS

The means and variances of dry weights of shoots and of the quantities of the three minerals are given in table 1. The data are also presented in the graphs in figs. 1, 2 and 3.

In analysing and interpreting these results it is necessary to pay special attention to two aspects of variation that are immediately apparent from the table and graphs. First, it will be seen that dry weights vary between families. Second, the total mineral content, as we should expect, increases with weight. It follows, therefore, that while obvious family differences in mineral composition are revealed by the seedlings these differences could, on the face of it, reflect merely

TABLE 1

*Means* ($\bar{\alpha}$), *Variances* (*V*) *and co-variances* (*W*xy) *of mineral contents* (y) *and dry weights* (x) *of seedlings in three rye families. Numbers of plants analysed are given in brackets*

| Genotype | Mineral | $\bar{\alpha}y$ *mgms.* | V*y* | $\bar{\alpha}x$ *mgms.* | V*x* | W*xy* |
|---|---|---|---|---|---|---|
| | | | < | > | | |
| *P6* (12) . . | K | 3·30 | 0·243 | 93·27 | 157·22 | 5·53 |
| *P17* (17) . . | | 3·66 | 0·565 | 122·94 | 589·32 | 17·27 |
| *F2* (18) . . | | 4·23 | 1·102 | 128·22 | 1015·46 | 32·22 |
| *P6* (12) . . | P | 0·36 | 0·004 | 93·27 | 157·22 | 0·71 |
| *P17* (17) . . | | 0·50 | 0·010 | 122·94 | 580·32 | 2·14 |
| *F2* (18) . . | | 0·55 | 0·010 | 128·22 | 1015·46 | 3·03 |
| *P6* (12) . . | Fe | 0·018 | 0·000009 | 93·27 | 157·22 | 0·03 |
| *P17* (16) . . | | 0·017 | 0·000025 | 116·42 | 617·72 | 0·10 |
| *F2* (17) . . | | 0·017 | 0·000008 | 127·75 | 1074·71 | 0·06 |

Although co-variances (W*xy*) are given for each mineral the relation between phosphorus and weight is in fact curvilinear, not linear (see text).

a variation in growth rate between the genotypes. What we need to know, however, and what must determine the statistical approach to analysing the data is whether genotypes vary in mineral composition independently of their growth rates. The method and the results of the analysis appear below.

(a) *Potassium.* The average potassium values for families, which are given in the table, differ significantly from one another, but, as mentioned above, these values are also correlated with the variation in average weight of families. The correlation is illustrated clearly in fig. 1, which shows the general increase in potassium associated with increasing weight.

To determine, now, whether family differences in potassium content exist independently of weight we note two features of the graph that are of special significance. First, the relation between the two characters within each family is linear—the joint regression being significant at the 0·1 per cent. level. Second, an analysis of variance shows no significant heterogeneity between the regressions in *P6*, *P17* and

the *F2*. The *b* values are, respectively, 0·035, 0·030 and 0·032. These two facts establish that the rate of change of potassium content on weight is consistent within families over the range of seedling weights represented. Consequently, by removing that part of the variation dependent on weight, which is common to all families, we can compare the families in respect of the residual variation in potassium that is

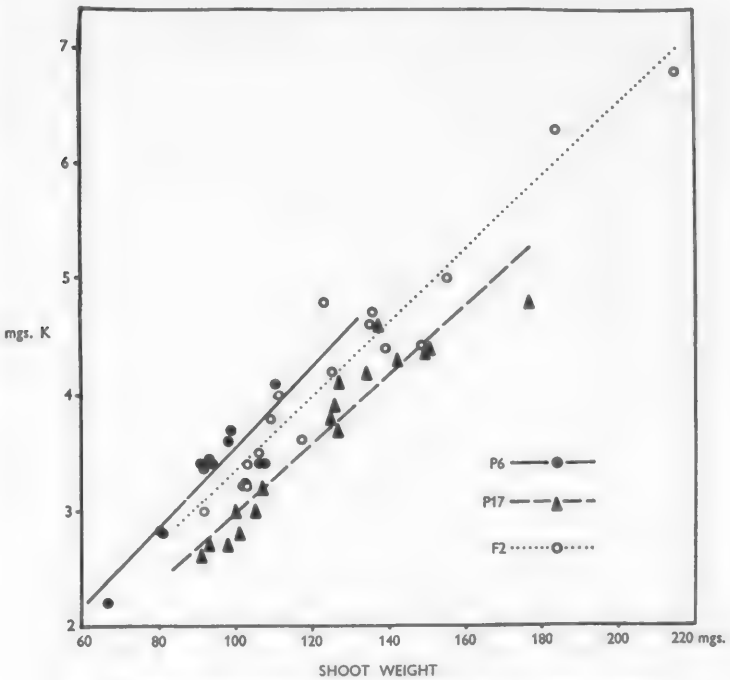


FIG. 1.—Potassium content of shoots of rye seedlings plotted against weight.

independent of seedling weight. A co-variance analysis serves the purpose admirably.

The analysis is set out in table 2. As will be seen the residual variation between genotypes is highly significant ($P = <0{\cdot}001$). For corresponding weights, as the graph shows, *P6* has the most, *P17* the least, potassium in its seedlings.

(b) *Phosphorus*. The graph in fig. 2 indicates a pattern of variation for phosphorus which in many respects is similar to that described for potassium. There is an increase in amount with increasing weight and there appear to be differences in phosphorus quantities for plants with corresponding weights from different families. In one respect, however, the phosphorus graph differs from the potassium one. While

the latter shows a linear increase in mineral quantity with increasing weight, that for phosphorus indicates a curvilinear relation with weight. A polynomial regression analysis of the data confirms an

TABLE 2

*The co-variance analysis of potassium content* (y) *and seedling weight* (x)

| | SS*x* | S*xy* | SS*y* | SS regr. | SS res. | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Total . | 37,893·31 | 1100·15 | 37·11 | 31·94 | 5·17 | N | MS | VR | P |
| Genotypes | 9,615·88 | 215·23 | 6·66 | ... | 2·41 | 2 | 1·21 | 20·17 | 0·001 |
| Error . | 28,277·43 | 884·92 | 30·45 | 27·69 | 2·76 | 43 | 0·06 | ... | ... |

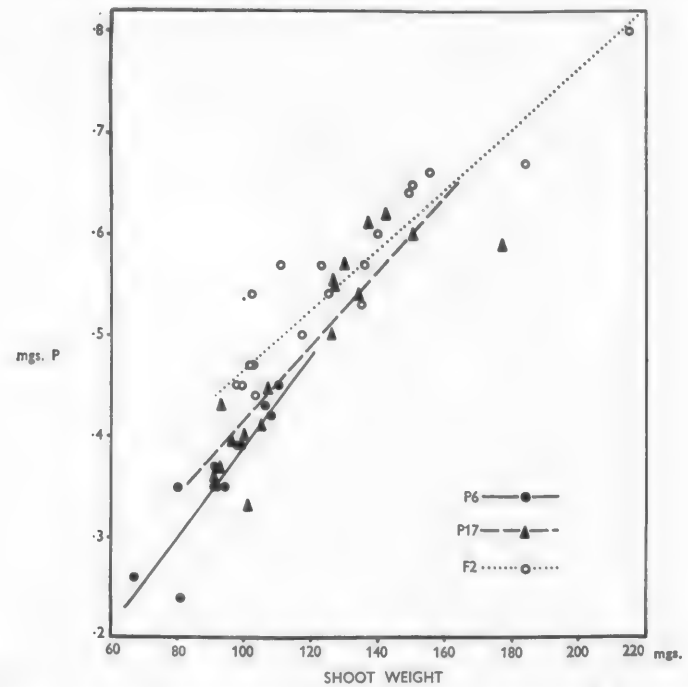


FIG. 2.—Phosphorus content of shoots of rye seedlings plotted against weight. For convenience the linear regressions have been plotted, whereas the relation between mineral and weight is in fact curvilinear (see text).

overall curvilinearity ($P = <0{\cdot}01$). In view of this it was clearly necessary to take curvilinearity into account when analysing, by co-variance, the phosphorus data. In the analysis, table 3, the

regression sums of squares consequently include both linear and quadratic items.

It will be seen from the analysis that phosphorus content, over and

TABLE 3

*The co-variance analysis of phosphorus content* (y) *on seedling weight* (x). *The regression sums of squares include both linear and quadratic components (see text)*

| | SS*y* | SS regr. | SS res. | | | | | |
|---|---|---|---|---|---|---|---|---|
| Total . . | 0·6427 | 0·5632 | 0·0795 | N | MS | VR | P | |
| Genotypes . | ... | ... | 0·0148 | 8 | 0·0074 | 4·93 | 0·02 | —0·01 |
| Error . . | 0·3888 | 0·3241 | 0·0647 | 42 | 0·0015 | ... | ... | ... |

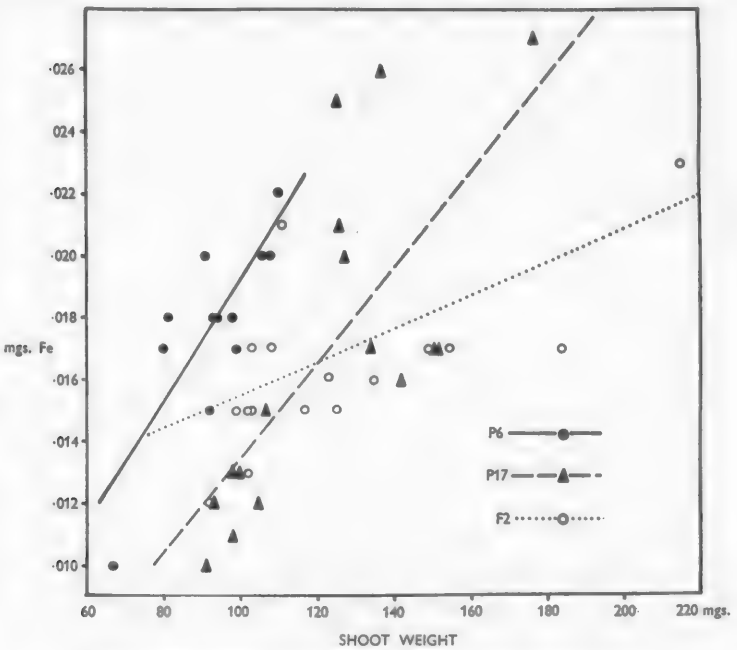


FIG. 3.—Iron content of shoots of rye seedlings plotted against weight.

above that accounted for by regression is significantly different between families.* For corresponding weights (fig. 2) *F2* seedlings contain the most phosphorus, *P6* seedlings the least.

* It is worth emphasising that in assessing and comparing the mineral compositions of plants the use of percentages, as is often adopted, to express content can be grossly misleading. This is especially true where content varies in curvilinear relation with weight. In such cases, differences in percentage values may reflect merely differences correlated with varying rates of development.

(c) *Iron.* The situation for iron (see fig. 3) is rather less straightforward than for either of the two minerals above. There is a similarity with potassium in that *within* families iron increases more or less linearly with increasing weight, and the joint regression is significant ($P = <0{\cdot}01$). In the case of iron, however, the regression slopes of families unlike those for potassium are very different from one another ($P = <0{\cdot}01$). In *P6* $b = 0{\cdot}00020$, in *P17* 0·00015 and in the *F2* 0·00006.

In view of this inconsistency between families an analysis of covariance is clearly inappropriate for the data as a whole. The graph in fig. 3 does, however, suggest how the data can be partitioned for separate comparisons. It will be seen that for *P6* and *P17* the regression slopes are very similar and, by analysis of variance, not significantly heterogeneous. On the strength of this a covariance analysis was therefore applied to the data for the two lines separately (table 4).

TABLE 4

*The co-variance analysis of iron content* (y) *on seedling weight* (x) *in* P6 *and* P17

| | SS*y* | S*xy* | SS*y* | SS regr. | SS res. | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Total . . | 16,923·08 | 1·4155 | 0·000479 | 0·000118 | 0·000361 | N | MS | VR | P |
| Genotypes . | 5,927·88 | −0·3500 | 0·000009 | ... | 0·000174 | 1 | 0·000174 | 24·86 | 0·001 |
| Error . . | 10,995·20 | 1·7655 | 0·000470 | 0·000283 | 0·000187 | 25 | 0·000007 | ... | ... |

From it we may conclude, as for the other minerals, that there is significant variation in iron content between genotypes of corresponding weights ($P = <0{\cdot}001$).

A second comparison, between *P17* and the *F2*, reveals a different sort of variation. Both families represent a similar range of seedling weights and their average iron contents are identical. The regression slopes, however, are significantly different ($P = 0{\cdot}02$-$0{\cdot}01$). It follows that not only does iron content vary independently of weight but also the rate at which it varies is apparently determined by the genotype.

To summarise, the results for iron confirm that the genotype determines variation in mineral composition of seedlings at comparable weights. They go further in suggesting that the rate of increase of mineral content which is to be expected with increasing weight is also determined genetically and may vary between genotypes.

## 4. DISCUSSION

The present evidence reveals heritable variation in the mineral composition of plants derived from the same initial population. There is every reason to suppose, therefore, that the nature of this variation

in populations will depend upon and will respond to the influence of selection.

What this variation signifies in physiological or developmental terms is not clear. One may suggest three main, not necessarily exclusive, possibilities.

1. The requirement for a particular mineral during a given increase in plant weight is greater in some genotypes than others, as Lyness has established in maize (1936).

2. The mineral requirement is the same but the mineral may be accumulated, unused, to a greater degree in different genotypes, as a result, for example, of differences in rates of mineral absorption (see Rabideau, Whaley and Heimsch, 1950).

3. Developmental differences from the morphological standpoint determine the differences in mineral composition, particularly where the balance of organs and tissues, which themselves have characteristic mineral compositions, are highly dissimilar. It is worth noting in this connection that although the rye seedlings investigated represent outwardly the same, three tiller, stage of differentiation and development the classification is, at best, a crude one, and even if we assume an identical morphology for the seedlings in all three families, at a cellular level their varying mineral composition may well be related to, and even determine, morphological divergence at a later stage of development.

Such possibilities need to be, and can be, tested. For the present, however, one can but reaffirm the heritability of plant mineral composition and emphasise that even within a population there are possibilities for change by selection, possibilities that no doubt play a prominent part in the adaptation of natural populations and which may, perhaps, play a greater part in plant breeding practice.

## 5. SUMMARY

1. Comparisons of potassium, phosphorus and iron content were made between seedlings of three rye families, two inbred lines and an *F2*, derived from the same commercial variety.

2. In all families the total mineral content of the seedlings increases with weight. For potassium and iron the increase is linear, for phosphorus, curvilinear.

3. There is evidence that the *rate* of change of iron content with weight is different in different families.

4. For all three minerals the content varies between different genotypes of corresponding weights. The significance of such heritable variation is briefly discussed.

*Acknowledgment.*—We are greatly indebted to Dr Alan Durrant for his help with the statistics.

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# THE EFFECT OF GAMMA IRRADIATION ON THE SEX RATIO OF *MELANDRIUM* SPECIES

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Received 24.i.61

THE data available on the effect of ionising radiations on sex ratios in higher organisms are conflicting. Early work with *Drosophila* showed that the sex ratio tended to change in favour of males (reviewed by Catcheside and Lea, 1945), and Schull and Neel (1955) concluded that there was a significant increase in the number of males in the progenies of human males exposed to the Japanese explosions. However, with mice exposed to doses of up to 1000 rads in both the pre- and post-sterile periods there was no significant change in the sex ratio of their progenies (Hertwig, 1938; Russell, 1954; Kohn, 1960). In the present experiment we have studied the effect of gamma radiation on the sex ratio of progenies of *Melandrium* species. These are dioecious species having an X-Y sex mechanism, a heterogametic male sex and heteromorphic sex chromosomes, the Y being longer than the X and having a definite male influence.

## 1. MATERIALS AND METHODS

Three populations of *Melandrium album* (*La* 6, 8 and 9) and one of *M. rubrum* (*La* 3) were used, one male plant and two females being chosen at random from each population. Two flowers on each female plant were pollinated with pollen from the selected male parent of that population. Mature pollen was exposed to one of the following doses of $\gamma$-rays, at a rate of 25 rads per minute—25, 100, 400, 1600 and 6400 rads, and used immediately afterwards for pollination of the female flowers. Control pollinations were also made in all instances. Care was taken to ensure that all pollinations were as uniform as possible—all being massive pollinations of the base of the styles. This was important in view of the known differential growth rates of X and Y bearing pollen tubes. All progenies were space planted in boxes maintained initially in the greenhouse and later outdoors.

## 2. RESULTS

Details of percentage germination and percentage females are given in tables 1-4. No correlation existed between these two percentages ($t_{10} = 1{\cdot}615$; $P = 0{\cdot}2$ to $0{\cdot}1$). The populations differed in terms of the percentage females and differences also occurred between genotypes within populations (table 5). In most instances there was a tendency for an increase in the proportion of females with increasing dose. This increase was not linear—a proportionally greater one occurring at lower doses, followed in several instances by a drop and a further later increase (see table 5 " within irradiated "). Control progenies differed

TABLE 1

| Dose in rads | Seeds | Germination % | Females in progeny % |
|---|---|---|---|
| *La 9 (2) × La 9 (4)* | | | |
| 0 | 626 | 19·0 | 30·3 |
| 25 | 621 | 21·4 | 51·5 |
| 100 | 580 | 54·8 | 58·9 |
| 400 | 892 | 5·8 | 42·3 |
| 1600 | 726 | 10·9 | 39·2 |
| 6400 | 721 | 21·1 | 61·8 |
| *La 9 (3) × La 9 (4)* | | | |
| 0 | 444 | 25·2 | 59·8 |
| 25 | 347 | 70·0 | 64·6 |
| 100 | 507 | 28·2 | 65·7 |
| 400 | 572 | 54·0 | 58·6 |
| 1600 | 507 | 48·7 | 58·7 |
| 6400 | 282 | 34·0 | 77·0 |

TABLE 2

| Dose in rads | Seeds | Germination % | Females in progeny % |
|---|---|---|---|
| *La 6 (3) × La 6 (4)* | | | |
| 0 | 403 | 28·0 | 84·6 |
| 25 | 108 | 57·4 | 83·3 |
| 100 | 409 | 74·6 | 79·8 |
| 400 | 429 | 35·4 | 75·4 |
| 1600 | 551 | 35·0 | 61·6 |
| 6400 | 475 | 20·0 | 80·9 |
| *La 6 (1) × La 6 (4)* | | | |
| 0 | 527 | 38·7 | 75·5 |
| 25 | 473 | 85·8 | 77·2 |
| 100 | 474 | 39·7 | 67·6 |
| 400 | 444 | 18·7 | 77·2 |
| 1600 | 470 | 31·1 | 81·9 |
| 6400 | 480 | 26·0 | 80·6 |

TABLE 3

| Dose in rads | Seeds | Germination % | Females in progeny % |
|---|---|---|---|
| *La 8 (2)* × *La 8 (1)* | | | |
| 0 | 590 | 38·8 | 71·9 |
| 25 | 514 | 32·5 | 76·7 |
| 100 | 365 | 40·0 | 85·5 |
| 400 | 502 | 33 3 | 75·7 |
| 1600 | 636 | 26·9 | 73·7 |
| 6400 | 533 | 34·7 | 77·6 |
| *La 8 (4)* × *La 8 (1)* | | | |
| 0 | 488 | 11·3 | 58·8 |
| 25 | 622 | 13·3 | 68·1 |
| 100 | 358 | 13·7 | 82·1 |
| 400 | 397 | 15·6 | 85·3 |
| 1600 | 494 | 9·1 | 78·8 |
| 6400 | 207 | 23·2 | 80·0 |

TABLE 4

| Dose in rads | Seeds | Germination % | Females in progeny % |
|---|---|---|---|
| *La 3 (3)* × *La 3 (1)* | | | |
| 0 | 597 | 42·2 | 52·4 |
| 25 | 622 | 31·8 | 55·0 |
| 100 | 525 | 56·6 | 58·1 |
| 400 | 518 | 48·8 | 58·9 |
| 1600 | 493 | 51·1 | 56·8 |
| 6400 | 549 | 29·5 | 62·4 |
| *La 3 (4)* × *La 3 (1)* | | | |
| 0 | 482 | 42·3 | 52·2 |
| 25 | 465 | 54·6 | 54·7 |
| 100 | 127 | 55·9 | 57·4 |
| 400 | 447 | 46·5 | 54·3 |
| 1600 | 434 | 43·8 | 58·4 |
| 6400 | 430 | 49·1 | 62·3 |

TABLE 5

*Analysis of variance for sex-ratio (per cent. females transformed to angles)*

| Item | M.S. | N. | V.R. | P. |
|---|---|---|---|---|
| Doses | 115·79 | 5 | 3·875 | 0·01-0·001 |
| (i) Control *v.* irradiated | 163·50 | 1 | 5·472 | 0·02-0·01 |
| (ii) Within irradiated | 107·65 | 4 | 3·603 | 0·02-0·01 |
| Populations | 1209·83 | 3 | 6·725 | 0·05-0·02 |
| Within populations | 179·91 | 4 | 6·021 | <·001 |
| Populations × dose | 42·32 | 15 | 1·416 | N.S. |
| Residual | 29·88 | 65 | ... | ... |

significantly from irradiated progenies, but no dose × population or dose × within population interaction existed. The last two items were bulked with replicate differences as the error component in the analysis. Since all populations responded in a similar manner, the data were bulked and the overall means are plotted against dose in fig. 1.

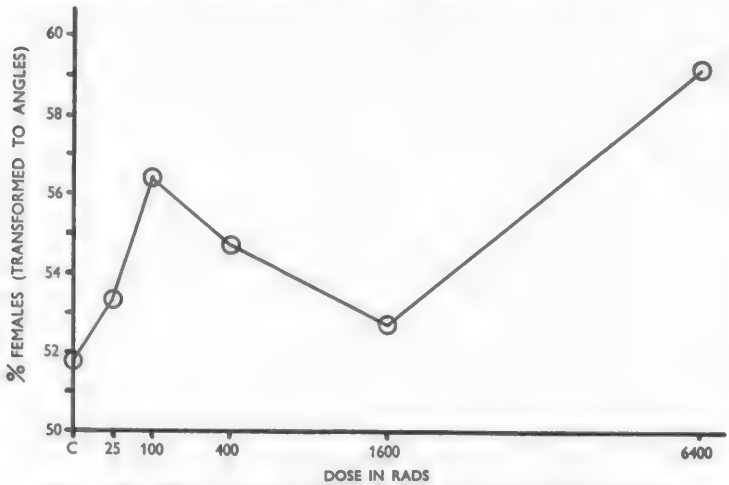


FIG. 1.—The effect of pollen irradiation on the sex ratio of *Melandrium* progenies.

## 3. DISCUSSION

A genetic control of sex ratio in *Melandrium* species has been demonstrated by Lawrence (1959), and the between and within population differences observed in the present instance may be understood in these terms. The magnitude of the differences in sex ratio was as great in some instances within as between populations. After exposing mature pollen to gamma rays an overall increase occurred in the percentage of female progeny surviving to maturity. This increase was marked at the lowest doses and could be attributed to physiological changes induced in the pollen grains which resulted in a more rapid

growth of X-bearing pollen tubes. These are known to have a faster growth rate than Y-bearing ones, and this natural advantage may have been enhanced by low doses of radiation. Such a stimulation of pollen tube growth has been reported by other workers (Swaminathan and Murty, 1959). At higher doses this presumably has not occurred, but another effect has been produced by the radiation involving the greater induction of lethality in male embryos. This may be understood in terms of the induction of lethal mutations in the sex chromosomes. In *Melandrium* the Y is longer than the X, and is physiologically active (Westergaard, 1958). It should therefore be as susceptible as the X per unit length of chromosome to the induction of dominant and recessive lethal types of injury. More dominant and recessive lethals would therefore be produced in the Y than the X. The former factor, dominant lethality, would lead to a greater lethality of male progeny as also would the latter as some recessive lethals would be expressed in the heterogametic sex.

These results do not agree with those obtained from analyses of mice, human, and *Drosophila* populations. With the last, an increase in males occurred, whereas with the first of these species no change in sex ratio has been observed after radiation exposures and the significance of the differences quoted by Schull and Neel (*loc. cit.*) between irradiated and control human populations has been queried by Kohn (*loc. cit.*). He has also contested the theoretical basis of their argument that an increase in the number of males might have been expected on the basis of the physiological inertness of the human Y chromosome, and hence its lack of susceptibility to a dominant lethal type of injury. Recent evidence would argue against such inertness of the Y (Jacobs and Strong, 1959; Ford, Polani, Briggs and Bishop, 1959). Some explanation of the variations in the response of different species may be found in the relative activities and sizes of the sex chromosomes. Humans and mice have a Y chromosome which is active, having a positive masculine effect, but which is smaller than the X. Because of this size difference, more dominant lethals should be produced in the X chromosome and an increase in males should occur. Recessive lethals would be unimportant here as we assume that they would not be expressed in the heterogametic sex. In *Drosophila* the Y is longer than the X, and on the basis of length more dominant and recessive lethality would be expected in the Y and hence more females would be expected in the progeny. However, the Y is considered to be relatively inert and less prone to this form of injury, and hence more males are found in the progeny of irradiated males. Of interest in this connection is the observation that though the totally heterochromatic Y chromosome of *D. viridis* is as long as the X, it participates in exchanges only half as frequently as the X (Baker, 1949).

*Acknowledgment.*—We are grateful to Professor Mather for his comments on the script.

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# EVOLUTION OF BARRIERS TO CROSSING OF SELF-INCOMPATIBLE WITH SELF-COMPATIBLE SPECIES OF *SOLANUM*

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## 1. INTRODUCTION

ATTEMPTS that have been made to cross a number of self-incompatible plant species with their self-compatible relatives have lead to the conclusion that in some cases the cross can be made only when the self-compatible species is used as the female. When the self-compatible parent is used as male the pollen tube fails to grow down the style of the self-incompatible pistillate parent. The most thoroughly documented examples of this phenomenon have been recorded for species of *Nicotiana* (Anderson and DeWinton, 1931), *Petunia* (Mather, 1943), *Lycopersicon* (McGuire and Rick, 1954), *Solanum* (Garde, 1959) and *Antirrhinum* (Harrison and Darby, 1955), species occurring in the related families *Solanaceæ* and *Scrophulariaceæ*. In addition, intergeneric crosses between members of the *Solanaceæ* and even inter-family crosses between members of the two families have been found to show this one-way crossing inhibition (Lewis and Crowe, 1958).

Numerous exceptions to the pattern are known in the form of successful self-incompatible × self-compatible crosses. Examples can be cited in, among other genera, *Lycium* (Lewis and Crowe, 1958), *Antirrhinum* (Harrison and Darby, 1955), *Papaver* (Fabergé, 1944), *Nicotiana* (East, 1919), *Brassica* (Kakizaki, 1930), *Layia* (Clausen, 1951), and *Potentilla* (Clausen and Hiesey, 1958). It has been hypothesised by Lewis and Crowe (1958) that the self-compatible parent is, in these cases, of recent origin, in its general genetic make-up is closer to the self-incompatible than the self-compatible group, and so in its crossing behaviour acts as a self-incompatible form.

Since the failure of the self-incompatible × self-compatible crosses may constitute a basic mechanism contributing to species isolation, it is important to determine how widespread it is in occurrence, and the nature of exceptions to its operation. The present study is an analysis of cross-ability of self-incompatible with self-compatible species of *Solanum*. In the course of this work, an example of reciprocal cross-ability between the two types was found that cannot be readily interpreted as resulting from a recent evolution of the self-compatible species from a self-incompatible progenitor.

In this analysis self-incompatible species of the series Tuberosa

* Paper number 264 from the Department of Botany and Plant Pathology, The Pennsylvania State University. This study was supported by a grant from the National Science Foundation.

and Commersoniana were crossed with clones of the self-compatible species *S. verrucosum* of the series Demissa. While the genetic mechanism responsible for self-incompatibility has not been determined for all of the species used, it has been established (Pal and Nath, 1942; Nath, 1942, 1945) that some members of the Commersoniana have oppositional alleles of the *Nicotiana* type. Recent evidence has been produced (Garde, 1959) showing that one-way isolation does occur between *S. verrucosum* and some members of the Tuberosa, although the cross *S. chacœnse* and *S. phureja* × *S. verrucosum* has been accomplished (Buck, 1960).

## 2. MATERIALS AND METHODS

Diploid species of *Solanum* of the section Tuberarium were used in the experiment. In the preliminary 1956-58 crosses listed in table 1, flowers were emasculated and

TABLE 1

*Results of preliminary crossings between diverse species of* Solanum *and* S. Verrucosum

| Self-incompatible species | Self-compatible *S. verrucosum* used as | | | |
|---|---|---|---|---|
| | Pistillate parent | | Pollen parent | |
| | No. pollinations | Berries set | No. pollinations | Berries set |
| Series Commersoniana | | | | |
| *S. chacœnse* | 26 | 14 | 26 | 11 |
| *S. chacœnse* spp. *subtilius* | 51 | 25 | 69 | 1 |
| *S. kurtzianum* | 6 | 2 | 13 | 0 |
| *S. tarijense* | 5 | 1 | 11 | 0 |
| Series Tuberosa | | | | |
| *S. phureja* | 19 | 2 | 19 | 0 |
| *S. stenotomum* | 34 | 12 | 36 | 0 |
| *S. soukupii* | 15 | 3 | 7 | 0 |
| *S. simplicifolium* | 15 | 6 | 23 | 0 |
| *S. neohawkesii* | 13 | 8 | 6 | 0 |
| *S. sparsipilum* | 9 | 2 | 4 | 0 |

pollinated at a late bud stage and protected from insect visit by the use of pieces of soda straw having an inner diameter of 8 mm. The species used for the 1959 crossings are listed in table 2 with their U.S. Department of Agriculture accession numbers and places of origin. For the 1959 crosses, flowers were emasculated in a late bud stage, allowed to open inside a cheesecloth bag, pollinated when open, and then covered again with the cheesecloth bag. This was done to eliminate the possibility that berry set could occur because pollinations were at a bud stage instead of on open flowers. All crossings were carried out in the greenhouse during winter and sprin gmonths under relatively constant environmental conditions.

## 3. RESULTS

Berry sets following preliminary reciprocal crossings between *S. verrucosum* and a number of species of the series Tuberosa and Commersoniana are listed in table 1. Berries were set when self-compatible

*S. verrucosum* was the pistillate parent, but the reciprocal crosses usually failed. This was in agreement with the model described above.

The successes of reciprocal crosses between *S. verrucosum* on the one hand and *S. chacœnse* and *S. chacœnse* ssp. *subtilius* on the other, however, disagree with the model. As a check against the possibility that pollen other than that of *S. verrucosum* had been used, some of the seeds of the *S. chacœnse* × *verrucosum* berries were germinated. The plants produced thereby were fertile $F_1$ hybrids whose morphology confirmed their identity. The most likely interpretation of the success of this cross seemed to be that different clones of *S. chacœnse* differ in their self-incompatibility, and that in those cases in which *S. chacœnse* styles

TABLE 2

*Identity and place of origin of clones used*

| Species | PI numbers | Place of origin |
|---|---|---|
| *S. chacœnse* . . | 189221(R), 230581(T), 133073(AA), 133619(AB), 133664(AC), AB × AC(IV), 195184(AD-1), 195184(AD-2) | Unknown Central Argentina |
| *S. chacœnse* ssp. *subtilius* . | 175443(O), 189217(CA), 189218(CF), 133085(CH), 133709(CI), 133722(CJ), 217451(CZ), 217451(DA), CA × CH(MT-6) CA × CH(MT-8) | Northwestern Argentina |
| *S. chacœnse* subspecies hybrid | AA × CF(IT-6), AA × CF(IT-7) | |
| *S. kurtzianum* . . | 133687(AU), 175434(AW) | Western Argentina |
| *S. simplicifolium* . | 208866(CK), 208866(CL-1), 218223(CN) 218224(CO-1), 218224(CO-2) | Northwestern Argentina |
| *S. soukupii* . . | 218227(CR-1) | Southern Peru |
| *S. stenotomum* . . | 195213(DL-2) | Central Peru |
| *S. verrucosum* . . | 195170(DI-2), 160228(FX), 195170(FZ), DI-1 × DG-1(QR), DI-1 selfed (QS), DI-2 × FZ-2(AJN) | South Central Mexico |

accepted *S. verrucosum* pollen tubes self-compatible clones of *S. chacœnse* had been used. Crosses were carried out in the winter and spring of 1959 to determine whether this was the case. Results of self-pollination of the clones and of pollination by *S. verrucosum* are listed in table 3.

Twenty-one of the 29 clones tested by over 20 separate self-pollinations per clone failed to set any berries at all, and the other 8 clones formed only 1 to 3 berries. If any of the clones tested in this way had been self-compatible, a berry set of over 50 per cent. such as is obtained when *S. verrucosum* is selfed would have been expected. The low set following self-pollination could not have resulted from female sterility, for in earlier tests (Grun, 1961) cross-pollination of these same clones succeeded in over 55 per cent. of the attempts made. Therefore, the few berries formed by 8 of the clones can probably be ascribed to pseudo-incompatibility.

Sixteen of these self-incompatible clones (table 3), agreed with the model in that they would not accept the self-compatible *S. verrucosum* pollen, while 12 disagreed with the model and showed a set of berries.

TABLE 3

*Results of self-pollination and pollination with self-compatible* S. verrucosum

| Species and clone | Self-pollinations | | Pollinations with *S. verrucosum* | |
|---|---|---|---|---|
| | No. attempts | Berries set | No. attempts | Berries set |
| *S. chacense* | | | | |
| R | 24 | [illegible] | 63 | 12 |
| T | 22 | 0 | 18 | [illegible] |
| AA | 22 | [illegible] | 70 | 16 |
| AB | 23 | 0 | 51 | 2 |
| AC | 23 | [illegible] | 61 | 15 |
| IV-6 | 22 | [illegible] | 64 | 3 |
| AD-1 | 22 | 0 | 41 | 15 |
| AD-2 | 23 | 2 | 60 | 33 |
| *S. chacense* spp. *subtilius* | | | | |
| O | 23 | [illegible] | 63 | 2 |
| CA | [illegible] | [illegible] | 60 | [illegible] |
| CF | 22 | [illegible] | 54 | 0 |
| CH | [illegible] | [illegible] | 47 | 2 |
| CI | 24 | 3 | 53 | [illegible] |
| CJ | [illegible] | [illegible] | 62 | [illegible] |
| CZ | 22 | [illegible] | 55 | 0 |
| DA-1 | 22 | 0 | 43 | [illegible] |
| MT-6 | 22 | 0 | 43 | 0 |
| MT-8 | 21 | 0 | [illegible] | [illegible] |
| *S. chacense* ssp. hybrid | | | | |
| IT-6 | [illegible] | 0 | 63 | [illegible] |
| IT-7 | 25 | [illegible] | 62 | [illegible] |
| *S. kurtzianium* | | | | |
| AU | [illegible] | [illegible] | 44 | [illegible] |
| AW | 25 | [illegible] | 42 | [illegible] |
| *S. simplicifolium* | | | | |
| CK | 24 | [illegible] | 34 | [illegible] |
| CL-1 | [illegible] | 0 | 32 | 0 |
| CN-4 | 22 | [illegible] | 57 | 0 |
| CO-1 | 23 | [illegible] | 40 | 0 |
| CO-2 | 24 | [illegible] | 67 | [illegible] |
| *S. soukupii* | | | | |
| CR-1 | [illegible] | [illegible] | 58 | 29 |
| *S. stenotomum* | | | | |
| DL-2 | 23 | [illegible] | 72 | 0 |

The presence or absence of pseudo-compatibility did not play any role in this regard, since 4 of the pseudo-compatibles would accept *S. verrucosum* pollen, and 4 would not. There was no particular indication that the pollen of the 6 separate lots of *S. verrucosum* used in these crosses

differed in effectiveness so that their separate data are summed in the table. Among the 12 self-incompatible clones that did set berries with *S. verrucosum* pollen, however, there were large differences, the per cent. set of different clones ranging from 2 per cent. to over 50 per cent. with a mean of 23 per cent. The difference in per cent. berry set of plants AB and AD-2, to take two extreme examples, is significant with a P value of less than $1 \times 10^{-8}$.

## 4. DISCUSSION

The primary purpose of this study was to determine whether the unidirectional barrier to crossing of self-compatible with self-incompatible plants operates between species of *Solanum*. In 16 of the tests made the unidirectional barrier did operate. This is a confirmation of the crossing results of Garde (1959). In 12 tests, however, the barrier was absent, and self-incompatible styles accepted the self-compatible pollen tubes of *S. verrucosum*. These results cannot be explained, as has been done for other exceptions (Lewis and Crowe, 1958) by saying that *S. verrucosum* is a recently formed self-compatible species whose genotype is primarily that of the self-incompatible forms. The fundamental nature of *S. verrucosum* as a self-compatible species is revealed by its inability to set berries on styles of the 16 other self-incompatible genotypes. These results have a direct counterpart in the variability of *Nicotiana alata* (Anderson and DeWinton, 1931), normal self-incompatible *N. alata* being receptive to the self-compatible pollen of *N. langsdorfii*, while *N. alata* 15-2 followed the unidirectional crossability pattern.

There are then different forms of self-incompatible plants, some of which accept self-compatible pollen, and some of which do not. How might these phenomena be interpreted in terms of evolution? Let us assume that from time to time self-compatible and self-incompatible populations meet in nature. A self-compatible population as a result of such a meeting would acquire genes for self-incompatibility and become more cross-pollinated. This change might result in an increase in its heterozygosity at the locale where the two forms met, but the previously self-compatible population would not become sterile or weak as a result of the meeting unless pollinating vectors were lacking. The meeting would also not affect the other populations of the self-compatible group since they, being isolated by their self-pollination mating system, would receive the self-incompatibility genes very slowly, if at all. The overall results of the meeting on the self-compatible complex would, therefore, be minimal.

The effects on the self-incompatible complex, however, could be more serious. The increased self-compatibility would lead to self-pollination with a resulting uncovering of recessive genes deleterious in the homozygous condition. The obligate cross-pollination, moreover, would lead to a spread of the self-compatibility genes in the

self-incompatible system, and so increase the likelihood of a general inbreeding degeneration. The self-incompatible system therefore would place a strong selection premium on any mechanism which resulted in exclusion of self-compatibility genes. It is precisely in this direction that the cross fails.

This barrier to introduction of self-compatibility genes has, however, broken down in 12 of the self-incompatible clones of *Solanum* tested. It seems possible that the breakdown of the barrier has occurred in the self-incompatible populations partially as a result of their geographic or ecological separation from all native self-compatible species of the genus. The self-compatible species of the Tuberarium section of *Solanum* in South America include *S. brevidens*, a native to South Central Chile and the Nahuel Huapi region of South Argentina, and *S. etuberosum* which is native to North Central Chile. The other three known self-compatible species of the section, *S. verrucosum*, *S. morelliforme* and *S. polyadenium*, are native to Central America (Hawkes, 1956). The biotypes of the present study which accepted *S. verrucosum* pollen include *S. chacœnse* from Central Argentina, one clone from North-western Argentina, and a clone of *S. soukupii* from Southern Peru. These all, therefore, lie outside of the range of their self-compatible relatives. The result of such separation would be that an internal mechanism operating against introduction of self-compatibility genes would no longer have a selective advantage. Selection for some other character could have resulted, as a by-product of pleiotropy or linkage, in loss of the unused barrier. An alternative, though perhaps unlikely, possibility is that these forms have never come into contact with self-compatible species of *Solanum* and so have not been under any selection pressure to develop isolation to crossing with them. The 16 self-incompatible clones that do not accept pollen tubes of *S. verrucosum* also are outside of the range of their self-compatible relatives. We must assume, if the hypothesis being suggested is correct, that these self-incompatible forms have an unused barrier against introduction of self-compatibility genes, which they have not as yet lost.

## 5. SUMMARY

The pollen of self-compatible *Solanum verrucosum* will not grow down the styles of some of the self-incompatible biotypes of several diploid species of *Solanum* although the reciprocal crosses regularly succeed. This result is in agreement with a general model for one-way isolation between self-compatible and self-incompatible species of plants. The one-way isolation is interpreted as resulting from a strong selection pressure for barriers which keep self-compatibility genes out of the heterozygous self-incompatible complex.

The same self-compatible *S. verrucosum* pollen does grow down the styles of other equally self-incompatible biotypes. This result is in disagreement with the general model for one-way isolation. The

biotypes which lack the one-way isolation occur outside the geographic range of self-compatible species of *Solanum*. Their acceptance of self-compatible pollen tubes is interpreted as resulting from a loss due to pleiotropy or linkage of their unused barrier against introduction of genes for self-compatibility.

*Acknowledgments.*—We wish to express our sincere thanks to Dr R. W. Hougas and the U.S. Department of Agriculture for supplying the plant material used in this study. We are also very much indebted to Drs H. G. Baker, J. L. Brewbaker and Jens Clausen for their critical readings of the manuscript.

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# SELECTION IN TWO ENVIRONMENTS

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Received 27.i.61

## 1. INTRODUCTION

A FREQUENTLY recurring problem of animal breeders is that of carrying out selection in the presence of interactions between genotype and environment. At one time it was widely believed that the only solution was to select different strains in every environment, so as to evolve one strain adapted to each (see, for example, Wright, 1939). However, Hammond (1947) suggested that animals should be selected for production " under environmental conditions which favour its fullest expression ", provided that the interactions were not of very great magnitude.

It was later shown by Falconer (1952) that there is no general solution to the problem, and that each case must be treated individually. Falconer treated the problem quantitatively by treating performances in two environments as genetically correlated characters, and using techniques already devised to deal with such characters. This concept has proved of great value in the interpretation of many experimental studies since reported and reviewed by McBride (1958).

It is the object of this paper to develop further some statistical aspects of the problem by extending Falconer's (1952) treatment to the case where genetic gain in both environments is desired, though the two environments may differ in importance. Such situations will arise particularly when only one or a few highly improved strains are to be used in a wide range of environments.

## 2. DERIVATIONS

Briefly, the approach initiated by Falconer is as follows. If $p_A$ and $p_B$ are the potential phenotypes of an individual if placed in environments $A$ and $B$ respectively, we write

$$p_A = g_A + e_A$$
$$p_B = g_B + e_B$$

where $g$ is the breeding value and $e$ the environmental effect. We then impose the restriction that the covariances of all terms on the right hand sides of these equations are zero, except that

$$E(g_A g_B) = r_G \sigma_{g_A} \sigma_{g_B}$$

where the $\sigma_g$'s are the genetic standard deviations. It is then readily

seen that if $\Delta_A g_A$ and $\Delta_B g_A$ are the respective gains in $g_A$ obtained by selection for $p_A$ and $p_B$, then we have

$$\Delta_A g_A = \tau h_A \sigma_{g_A}$$
$$\Delta_B g_A = \tau r_G h_B \sigma_{g_A}$$

where $\tau$ is the selection differential in standard deviations and $h_A^2$, $h_B^2$ are the heritabilities in the two environments. It is then clear that if $\Delta_B g_A > \Delta_A g_A$ we must have $r_G h_B > h_A$. There are similar results for $\Delta_B g_B$ and $\Delta_A g_B$, the meanings of which terms are obvious.

The question now arises as to how selection should be made if performance in neither environment is irrelevant. This is simply dealt with in the normal manner by letting a gain in $g_A$ have $w$ times the value of an equal gain in $g_B$. If then $\Delta_A g$ is the overall genetic gain by selection for $p_A$ we have at once

$$\Delta_A g = w\Delta_A g_A + \Delta_A g_B$$

and

$$\Delta_B g = w\Delta_B g_A + \Delta_B g_B.$$

Writing $\sigma_{g_A}/\sigma_{g_B}$ as $H$ for convenience, we have from above

$$\frac{\Delta_B g}{\Delta_A g} = \frac{h_B}{h_A}\left[\frac{1 + wHr_G}{wH + r_G}\right].$$

This is conveniently rewritten as

$$\frac{\Delta_B g}{\Delta_A g} = \frac{h_B}{h_A}\left[r_G + \frac{1 - r_G^2}{r_G + wH}\right]$$

for comparison with $\Delta_B g_A / \Delta_A g_A$. It can be seen that Falconer's result corresponds to the special case when $w = 0$. A point of interest here is that when $wH = 1$ the equation reduces to

$$\frac{\Delta_B g}{\Delta_A g} = \frac{h_B}{h_A}$$

and in this case the extent of the interaction between genotype and environment is irrelevant.

So far it has been assumed that only one environment is to be used, but it is possible to select two strains, one in each environment, and denoting the overall gain from this procedure as $\Delta g$, we have

$$\Delta g = w\Delta_A g_A + \Delta_B g_B.$$

From this it is easily shown that

$$\frac{\Delta_A g}{\Delta g} = 1 - \frac{1 - r_G \dfrac{h_A}{h_B}}{1 + wH\dfrac{h_A}{h_B}}$$

assuming that the intensity of selection is the same in both populations.

It then follows that $\Delta_A g > \Delta g$ if and only if $r_G h_A > h_B$, which result is of course as expected.

There is yet another approach, which gives rise to a different problem. The breeder may wish to test one strain in each of two environments so that it is adapted to both, and select on an index combining performances in both environments. An individual cannot usually be itself tested in both environments, but such procedures as progeny testing provide a suitable method. For instance, in progeny testing to improve egg production in the domestic fowl, groups of daughters from each tested cockerel may be divided, some being tested on farms with an endemic disease, others on farms where this disease is absent. Then the two " phenotypes " of a cockerel are the means of his progeny groups.

The selection index is taken to be of the form

$$I = kp_A + p_B$$

and the problem is to find the value of $k$ which maximises overall genetic gain as previously defined. The general method of solution is well known, *e.g.* Smith (1936) and Hazel (1943). The special conditions associated with the present problem provide a particular solution, and this turns out to be

$$k = \frac{wh_A^2}{h_B^2}\left[\frac{(1-r_G^2)+r_G(1-h_B^2)(r_G+\frac{1}{wH})}{(1-r_G^2)+r_G(1-h_A^2)(r_G+wH)}\right].$$

It should be remembered that in the application to a case such as outlined above the parameters in this equation are those associated with progeny groups. These are well-known (Lerner, 1958) and need not be discussed here.

The expression for $k$ is rather formidable and some special cases are now given in illustration. When $r_G = 0$ we have $k = w\frac{h_A^2}{h_B^2}$, the general result for uncorrelated characters. When $r_G = 1$ we have $k = H\sigma_{e_B}^2/\sigma_{e_A}^2$, and when $wH = 1$ we have

$$k = w\frac{h_A^2}{h_B^2}\left[\frac{1-r_G h_B^2}{1-r_G h_A^2}\right].$$

This last value will usually be very close to that for uncorrelated characters.

When $w = 0$ we can find that

$$k = \frac{h_A}{Hh_B}\cdot\frac{r_G h_A}{h_B}\cdot\frac{1-h_B^2}{1-(r_G h_A)^2}.$$

In the case where $\Delta_A g_B = \Delta_B g_B$ we have $r_G h_A = h_B$ and the equation becomes

$$k = \frac{h_A}{Hh_B} = \frac{\sigma_{p_A}}{\sigma_{p_B}}.$$

If index selection is to be preferred its greater operational difficulty must be repaid by considerable increases in genetic gain. If then $\Delta_{Ig}$ is the overall gain by index selection, we must compare this with $\Delta_{Bg}$ and $\Delta g$ as previously defined. The general expression for $\Delta_{Ig}$ is extremely complicated and the general comparisons have not been made. However, when $wH = 1$ the comparisons become much simpler and we may use this case as a guide to the general situation. Comparing then $\Delta_{Ig}$ with $\Delta_{Bg}$, it turns out that we have

$$\frac{\Delta_{Bg}}{\Delta_{Ig}} = h_B \sqrt{\frac{1-r_G^2 h_A^2 h_B^2}{h_A^2+h_B^2-2r_G h_A^2 h_B^2}}.$$

Now, assuming that $h_B^2 - h_A^2$ is small compared with $h_B^2$ we can write this approximately as

$$\frac{\Delta_{Bg}}{\Delta_{Ig}} = \sqrt{\frac{1+r_G h_B^2}{2}}.$$

It is thus unlikely that $\Delta_{Bg}$ will be more than about 80 per cent. as great as $\Delta_{Ig}$ so that index selection has a very considerable advantage.

It may be noted also that this is the approximate result obtained for $w = 0$ and $r_G h_A = h_B$ approximately.

Once more taking $wH = 1$ we have

$$\frac{\Delta_{Ig}}{\Delta g} = \frac{1+r_G}{h_A+h_B}\sqrt{\frac{h_A^2+h_B^2-2r_G h_A^2 h_B^2}{1-r_G^2 h_A^2 h_B^2}}$$

and using the same approximation as before we find that this reduces to

$$\frac{\Delta_{IG}}{\Delta_G} = \sqrt{\frac{(1+r_G)^2}{2(Hr_G h_B^2)}}.$$

From this it follows that index selection will usually be more efficient provided that $r_G > 0{\cdot}7$ approximately.

TABLE 1

*Values of* $\frac{k}{w}$ *for specified values of* $r_G$ *and* $wH$ *for* $h_A^2 = 0{\cdot}57$, $h_B^2 = 0{\cdot}51$

| $r_G$ | $wH$ | | | | |
|---|---|---|---|---|---|
| | ½ | 2/3 | 1 | 1½ | 2 |
| ·0 | 1·114 | 1·114 | 1·114 | 1·114 | 1·114 |
| 0·25 | 1·326 | 1·238 | 1·133 | 1·039 | 0·973 |
| 0·50 | 1·570 | 1·379 | 1·160 | 0·987 | 0·861 |
| 0·75 | 1·915 | 1·572 | 1·200 | 0·917 | 0·754 |
| 1·0 | 2·533 | 1·900 | 1·267 | 0·844 | 0·633 |

This would apply with progeny groups of size 20 with individual heritabilities of 0·25 and 0·20.

As a further illustration of the equation for $k$, tables 1 and 2 give the values of $k$ for some particular situations. In these tables it can be seen that over the range of $r_G$ from zero to one $k$ changes approximately linearly. Since it has been shown by Robertson (1959) that sampling errors in the estimation of $r_G$ are likely to be large, and all other parameters are also subject to errors of estimation, there may be little value

TABLE 2

*Values of* $\frac{k}{w}$ *for specified* $r_G$ *and* $wH$ *with* $h_A^2 = 0{\cdot}25$, $h_B^2 = 0{\cdot}20$

| $r_G$ | $wH$ | | | | |
|---|---|---|---|---|---|
| | ½ | 2/3 | 1 | 3/2 | 2 |
| 0·0 | 1·25 | 1·25 | 1·25 | 1·25 | 1·25 |
| 0·25 | 1·61 | 1·45 | 1·27 | 1·11 | 1·00 |
| 0·5 | 1·94 | 1·63 | 1·29 | 1·01 | 0·85 |
| 0·75 | 2·29 | 1·81 | 1·31 | 0·94 | 0·75 |
| 1·0 | 2·67 | 2·00 | 1·33 | 0·89 | 0·67 |

This would apply for progeny groups of size 20 and individual heritabilities of 0·066 and 0·050.

in extreme accuracy in the calculation of $k$ from these parameter estimates. It is therefore suggested that a satisfactory estimate of $k$ may be obtained by linear interpolation between $w\frac{h_A^2}{h_B^2}$ and $H\frac{\sigma_{eB}^2}{\sigma_{eA}^2}$ at the estimated value of $r_G$.

It may be worth pointing out that these results apply to correlated characters in general, provided these correlations are genetic only. This may be compared with the method of Rendel (1954) to deal with characters environmentally correlated.

Exactly the same method may be applied to selection in more than two environments, but there seem to be no useful simplifications such as arise in the case of two environments. It is simple to write out the equations requiring solution.

## 3. DISCUSSION

In recent years there has been a tendency for the means of genetic improvement of domestic animals to be concentrated in the hands of a few large breeders. This has been particularly marked in the poultry industry. Under such conditions, the ability of a breeder's stock to produce well over a wide range of environments becomes extremely important, and what has here been called overall genetic gain will be the object of selection programmes.

The simplest approach is of course to ignore genotype-environment interactions, and if this is done selection should be carried out in the

environment giving greater overall gain. For two environments this has been shown to depend on $h_A^2$, $h_B^2$, $H$, $w$ and $r_G$. Table 3 gives values of $h_B^2/h_A^2$ which must be exceeded for given values of $r_G$ and $wH$ if $p_B$ is to be selected for in preference to $p_A$.

Over what seems likely to be the most important part of the range $[2/3<wH<3/2: 0{\cdot}7<r_G<1]$ it can be seen that if one heritability is about 1·15 times as large as the other, this will be a sufficient increase to determine which environment should be used. For instance, if $wH = 2/3$, $r_G = 0{\cdot}7$, $h_A^2 = 0{\cdot}25$, then if $h_B^2$ is less than 0·217 selection should be for $p_A$. However, the differences in gains are small unless $h_B^2$ is much less than 0·217, and in practice the final decision would

TABLE 3

*Values of* $\frac{h_B^2}{h_A^2}$ *for which* $\Delta_{BG} = \Delta_{AG}$ *if* $\frac{h_B^2}{h_A^2} >$ *tabulated value,* $\Delta_{BG} > \Delta_{AG}$

| $r_G$ | $wH$ | | | | |
|---|---|---|---|---|---|
| | 1/3 | 2/3 | 1 | 3/2 | 2 |
| 0 | 0·250 | 0·444 | 1 | 2·250 | 4·000 |
| 0·2 | 0·404 | 0·585 | 1 | 1·711 | 2·468 |
| 0·4 | 0·562 | 0·709 | 1 | 1·411 | 1·777 |
| 0·6 | 0·716 | 0·819 | 1 | 1·221 | 1·397 |
| 0·8 | 0·863 | 0·916 | 1 | 1·092 | 1·160 |
| 1·0 | 1·000 | 1·000 | 1 | 1·000 | 1·000 |

usually be based on the operational convenience of the two environments.

It may often be desirable to avoid difficulties involved in the maintenance, testing and selection of two separate strains, especially when there are chances that the purchaser will use one strain in an environment different from that for which it was developed. Under these conditions it would seem best to develop a strain adapted to both environments, and the results given above suggest that a selection index will be the most efficient method of developing such a strain. However, the comparison given above of overall gains from index selection with those from separate selection will usually be biased in favour of the index. This follows from the assumptions that selection intensity is the same in both strains, and that population size is the same for each strain. These objections appear to be important only if $w$ deviates considerably from one, so the bias may not be very great in many cases.

It might appear that use of the index would necessitate the doubling of testing facilities, but this is by no means always correct. For instance, if poultry are tested on outside farms, it involves a splitting of these

farms into two groups and extra work in data processing, which does not double the operational problems.

One interesting result of the experiments of Falconer and Latyszewski (1952) and Fowler and Ensminger (1957) has been that selection in "optimal" (A) conditions has been less efficient than selection in "sub-optimal" (B) conditions. In such situations it seems in general that $h_A^2 > h_B^2$ and $H < 1$. We here consider a hypothetical case of this type. We take $h_A^2 = 0{\cdot}25$, $h_B^2 = 0{\cdot}20$, $H = 0{\cdot}7$, $w = 1$, $r_G = 0{\cdot}4$. Then we have $\frac{\Delta_{Bg}}{\Delta_{Ag}} = 1{\cdot}04$. Substitution of these values in the index equation gives the result $k = 1{\cdot}523$. The use of the suggested linear interpolation would yield $k = 1{\cdot}512$. It is noteworthy that in this example the phenotype which is less efficient alone is given the greater weight in the index. Such a result is by no means intuitively obvious.

When the actual comparison is made we find $\frac{\Delta_{Bg}}{\Delta_{Ig}} = 0{\cdot}7526$. Using the approximate formula given earlier we would obtain $\frac{\Delta_{Bg}}{\Delta_{Ig}} = 0{\cdot}734$. This example shows how the suggested approximations work in a particular case.

It should be noted that the present formulation differs from Falconer's (1952) in two respects. His path diagram may be written as

$$p_A = g_A + e_A + t_A$$
$$p_B = g_B + e_B + t_B$$

where the $t$'s are effects associated with specific environments, while the $e$'s are random variables within these environments. In the present model these have been amalgamated. A more important difference is the present assumption that $E(e_A e_B) = 0$ which is not made by Falconer. This does not affect the results except in the derivation of the index. However, in the situation discussed, where "phenotypes" are means of related groups, this assumption is simply that commonly made of no environmental correlations between relatives. For completeness we may include the equation for $k$ when $r_P \neq r_G h_A h_B$. It is

$$k = w\frac{h_A^2}{h_B^2}\left[\frac{(1+\frac{r_G}{wH})-(\frac{1}{wH}+r_G)r_P\frac{h_B}{h_A}}{(1+wHr_G)-(wH+r_G)r_P\frac{h_B}{h_A}}\right].$$

which clearly reduces to that given earlier when $E(e_A e_B) = 0$.

It is very doubtful whether the simple model presented here would give accurate predictions over an extended period. However, it seems probable that the index method may be of considerable value over a few generations, after which the selection procedure would need to be revised in the light of results.

## 4. SUMMARY

1. The " overall gain " through selection is defined when animals are to be used in two environments.

2. Three selection procedures are considered:

(i) Selection in one environment ;
(ii) Separate selection in both environments of two strains ;
(iii) Selection on an index combining performance in both environments.

3. Expected overall gain is worked out for each procedure, and the optimum index is derived.

4. Comparison of gains is made to give a basis for choosing the form selection should take. Over a range of conditions index selection is most efficient, producing greater overall gain than even separate selection.

5. The implications of these results are discussed.

*Acknowledgments.*—I wish to thank the Rural Credits Development Fund of the Reserve Bank of Australia for a Fellowship during the tenure of which this work was done. I wish also to thank Dr G. McBride for his helpful comments.

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# GENE STRUCTURE AND FUNCTION IN THE $h_{III}$ REGION OF BACTERIOPHAGE T4

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Received 6.ii.61

## 1. INTRODUCTION

FROM the strain of *Escherichia coli* K12S, a strain KS/4 can be isolated as a survivor from a plating of K12S with a large excess of T4D wild type phage (Edgar, 1958). The latter when plated on KS/4 give faint turbid plaques (ghost plaques) with a low efficiency of plating, *i.e.* less than 10 per cent. of the phage give a plaque. However, mutants (*h*) of the phage may be isolated which give clear plaques on KS/4 with close to the same efficiency of plating as on the sensitive strain K12S or S/6. These *h* mutants may be distinguished from wild type ($h^+$) on appropriate mixed bacterial platings. For example on mixtures of S/6 to K/4 in the ratio 5:1 the *h* mutants give clear plaques and the wild type $h^+$ produce turbid plaques with a clear edge. Thus the use of this mixed indicator allows the *h* mutants to be treated as a morphological marker for experimental purposes.

Using these procedures Edgar (1958) isolated eleven independent *h* mutants which gave clear plaques on the mixed indicator. From crossing the latter in all possible combinations he concluded that they occupied six closely linked sites. Further by crossing three of them to *m41* (a mutant giving minute plaques) and *tu45* (a mutant giving turbid plaques) Edgar showed that they occupied a region on linkage group III between *m41* and *tu45*. The recombination frequencies based on the relatively small progenies scored from the crosses did not allow the *h* mutants to be arranged in an unambiguous linear order.

The clustering of the *h* loci on a relatively short segment of linkage group III and the suggestion that there is "phenotypic mixing" between *h* and $h^+$ characters (Edgar, 1958) similar to that found by Novik and Szilard (1951) in phage T2, which might be adopted as a test of functional allelism (Streisinger and Franklin, 1956), makes these mutants potentially useful for investigating the relationship between gene structure and function. The present paper extends the type of analysis of structure and function carried out by Benzer (1955) on the $r_{II}$ region in phage T4B to the $h_{III}$ region of phage T4D.

## 2. MATERIAL AND METHODS

*Phages.* The *h* mutants used were those of Edgar's earlier work. Mutants of the *tu45* region which were also used were kindly supplied by Dr A. Doermann and described by Doermann and Hill (1953). The phage stocks, prepared by the method

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of Chase and Doermann (1958), were sterilised by the addition of chloroform followed by centrifugation and filtration.

*Bacteria.* *Escherichia coli* strain B was used as host for crosses. Strains S/6, a derivative of B (Doermann and Hill, 1953), and KS/4 were used as indicator strains for plating.

*Crosses.* Crosses were performed in the manner described by Chase and Doermann (1958) except that anti-serum was not used. Unadsorbed phage was determined by assay after shaking a sample from the adsorption mixture with chloroform. In no case was there more than 0·1 per cent. unadsorbed parental phage in the progeny of the crosses. In all crosses considered later in this paper the total parental multiplicities varied between 12 and 18 and in no case does the parental imput ratio vary significantly from 1 : 1.

## 3. RESULTS

### (i) *Mapping the* h *mutants*

Five of the original *h* mutants (*h41*, *h43*, *h49*, *h50* and *h52* of Edgar, 1958) were crossed together in all combinations and in view of their heat sensitivity, described later, all precautions were taken to avoid exposing the phages to temperatures above 5° C. longer than was absolutely necessary to perform the crosses. Similar precautions were exercised with their progeny. In these as in all other crosses described in this paper sufficient progeny were scored from each cross so as to include at least 100 recombinants.

Two recombinant genotypes are expected from these crosses, wild type and the reciprocal double *h* mutant, and while the phenotype of the first of these is known we have no reason to expect the double *h* mutant to be lethal, or to be like a single *h* or even wild type in phenotype on the indicator strains. Its phenotype was therefore determined as follows. From two of the crosses $h41 \times h43$ and $h41 \times h49$ twenty wild type recombinants were backcrossed to a known wild type strain. All the progeny were wild type. Had the double *h* mutant a wild phenotype approximately half these backcrosses would have segregated single *h* mutant recombinants. Unless the double *h* mutant is lethal it must, therefore, have a single *h* phenotype on the indicator strains. To distinguish between these two possibilities fifty *h* progeny from each of the crosses $h41 \times h43$, $h43 \times h49$ and $h43 \times h50$ were backcrossed to each of the two parents of the cross. Among the fifty *h* progeny from each of these three crosses 3, 2 and 2, respectively, failed to give wild type recombinants in either of the two backcrosses, although sufficient progeny were scored from the latter to give less than one chance in a thousand of missing the wild type recombinant (due to the vagaries of sampling) if present. We can conclude, therefore, that these anomalous *h*'s are in fact the double *h* mutants and further phenotypic classifications described later confirm this conclusion. Furthermore, the frequency with which the double *h* recombinant is recovered in these and later experiments is that expected from equality with the much larger samples of the wild type reciprocal recombinant. Since the

method of estimating the frequency of the double *h* recombinant class is clearly unsuitable for large scale determinations, twice the frequency of the wild type recombinant class has been used for mapping purposes. These frequencies allow the five *h* mutants to be ordered in the unambiguous linear sequence shown in fig. 1. This sequence has been confirmed in a number of ways. Firstly the five *h* mutants have been crossed to *tu45a* where both recombinant classes, namely *tu45h* and wild type can be scored on the indicator strains. These crosses gave the recombination frequencies given in fig. 1 and they are compatible with the previously determined linear sequence. Secondly the *tu45h* recombinant stocks obtained in this way were crossed to a different *h* mutant. This provides a simple qualitative test of the positions of

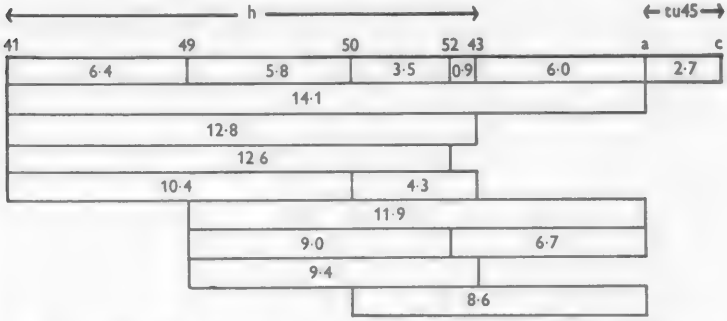


FIG. 1.—Linkage map of the *h111* region of phage T4D. All recombination frequencies are based on more than 100 recombinants from equal parental impact crosses with total multiplicities between 12 and 16.

the two *h* mutants relative to *tu45*. For example if *hA* is crossed to *hB tu45* and *hA* lies outside the *hB-tu45* region then the majority of $h^+$ recombinants will be non-turbid since turbids could only arise by simultaneous crossovers in both the *hA-hB* and *hB-tu45* regions. On the other hand, if *hA* lies between *hB* and *tu45* then the relative frequencies of turbid and non-turbid $h^+$ recombinants will be reversed. In this way *hA* and *hB* may be ordered relative to *tu45*. This test again confirms the order of the *h* mutants given in fig. 1.

### (ii) *Possible allelism*

On small samples of progeny Edgar (1958) found that four *h* mutants(*h44*, *h45*, *h46* and *h47*) failed to give wild type recombinants when crossed to *h41*, suggesting possible allelism. This tentative conclusion has been confirmed in so far as progenies have been examined large enough to rule out frequencies of wild type recombinants as low as $10^{-3}$ at which frequency the presence of reversions to $h^+$ in the parental stocks makes higher resolutions impossible (Jinks, 1961).

### (iii) *Adsorption of* $h_{III}$ *mutants to KS/4*

From the approximate rates of adsorption of *h43* and wild type to KS/4 (Edgar, 1958) it appears that the difference between the two is quantitative rather than qualitative, there being a measurable adsorption of the wild type phage. In a more detailed comparison the adsorption curves for *h41* and wild type have been determined. The adsorptions were carried out at 35° C. using $2 \times 10^7$ mixture of *h41*

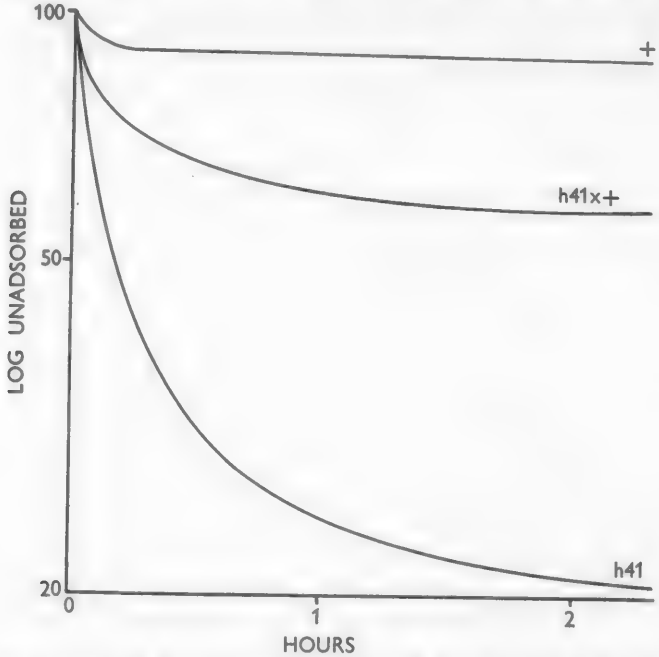


FIG. 2.—Graphs of the log percentage unadsorbed phage of mutant *h41* and wild type and the progeny of a cross between *h41* and wild type against time in hours using $2{\cdot}8 \times 10^8$ bacteria of strain KS/4 at 35° C. The graphs for the mutant (clear plaques) and wild type phage (turbid plaques) in the progeny of the cross are identical, hence there is "phenotypic mixing" for adsorption to KS/4.

and wild type in equal parts and $2{\cdot}8 \times 10^8$ log phase KS/4. At appropriate intervals the unadsorbed phage was assayed as for the crosses. Plotting the logarithm of the percentage of unadsorbed phage against time for *h41* and wild type phage in the mixture gives the graph in fig. 2. Both are curvilinear, an initial period of rapid adsorption being followed by a period of almost no adsorption. The approximate rate of adsorption during the first period is $0{\cdot}8 \pm 0{\cdot}29$ per cent. per minute for wild type and $2{\cdot}8 \pm 0{\cdot}47$ per cent. for *h41*. This rate becomes virtually zero after about 8 per cent. of wild type and 80 per cent. of *h41* phages have been adsorbed.

The fate of the adsorbed fraction of the phage appears to be similar for both wild type and mutant although the number of phage released per bacterium, *i.e.* the burst size, is 240 for wild type but only 130 for the mutant under the condition used for making the crosses. Before, however, paying too much attention to the absolute values obtained we must consider the heat sensitivity of the *h* mutants which would distort both their apparent rate of adsorption and their burst size as estimated here.

### (iv) *Heat sensitivity*

All the $h_{III}$ mutants and *tu45* mutants *a*, *b*, *c* and *d* of Doermann and Hill (1953) are inactivated at a faster rate than wild type phage at temperatures above 25° C. Since this difference in rate of inactivation is greatest at 45° C. all comparisons were made at this temperature. Apart from this no further attempts were made to find experimental conditions which maximised this difference as it was in general sufficient for our purposes.

The heat inactivations were invariably carried out in screw cap glass containers using 5 ml. of approximately $5 \times 10^6$ phage per ml. in EHA broth. Samples of 0·1 ml. were removed at appropriate intervals and diluted in chilled broth, the dilutions having been previously estimated from pilot experiments to give between 200 and 400 surviving phage per 0·1 ml. plated. Whenever possible the control wild type phage was mixed with the mutant in 1:1 proportions before inactivation and the inactivated mixture plated on an indicator strain on which both mutant and wild type could be distinguished, *i.e.* 5:1 S/6 and KS/4 for *h*/+ mixtures and S/6 for *tu45*/+ mixtures. Occasionally, because of difficulties of classification, an independent wild type control was used.

In general the inactivations were exponential for as far as $10^{-3}$ survival which more than covered the range over which we were primarily interested. Hence the logarithms of the percentage survival plotted against time are more or less straight lines (fig. 3). The graphs given in fig. 3 are based on as many as ten independent consistent inactivations and never less than two and each point on each occasion was based on a count of more than 100 survivors.

Somewhat more uniform slopes were obtained by inactivating single cycle stocks instead of the higher titre laboratory stocks produced by multiple cycling. The additional precisions which resulted from the single recycling was not considered worth the effort for the investigations in hand although it is clearly of importance in considering the mechanism of inactivation.

The log rates of inactivation of the $h_{III}$ mutants fall between 2·62 and 96·13 times the rate for wild type, the extreme mutants being *h48* and *h50* respectively. Mutant *h48* is included in these comparisons because it represents the lower extreme of heat sensitivity. It is not, however, included in the map given in fig. 1. Its exact location has yet

to be determined although it is within 1 per cent. recombination frequency of *h43* and it maps to the right of *h41*, *h49* and *h50*.

The turbid mutants of the *tu* region are about average in their sensitivity, giving rates of inactivation relative to wild type from 14·75 to 27·78. None of the other turbid loci in phage T4D, *i.e. tu41*, *tu42*, *tu43* and *tu44* of Doermann and Hill (1953) is more heat sensitive than wild type. Thus the only sensitive turbids are those which adjoin the sensitive $h_{111}$ region.

### (v) *The nature of the heat inactivation*

The heat inactivated phage are killed in the sense that they fail to give a " plaque " on an indicator strain of sensitive bacteria under the normal plating conditions. However, there are many stages between the successful attachment of a phage particle to a sensitive bacterium and the release of sufficient viable phage progeny to produce a plaque and a phage may be classified as dead as a result of its failure to complete any one of these stages following the heat treatment.

To obtain some indication of the stage at which this failure occurred heat inactivated phage were compared for their plaque forming and bacterial killing ability. A stock of *h41* was heat inactivated in the usual way for ½, 1, 2, 3 and 4 hours at 45° C. and at each time interval assayed for its plaque forming ability. At the same time intervals samples of the phage were assayed for their ability to kill bacteria by adding $8 \times 10^8$ of the treated phage to $4 \times 10^8$ bacteria at 35° C.

Both plaque forming ability and bacterial killing ability fell at identical exponential rates with increasing time of exposure of the phage to 45° C. Hence a phage which is dead on the basis of its failure to produce a plaque is also dead in the sense that it fails to kill a sensitive bacterium.

### (vi) *Phenotypic mixing*

In phage T2 the elements of the phage particle responsible for the specific adsorption of phage host cells are produced in a pool by the joint action of all the phage within the same bacterium. The specificity of the elements is determined by the *h* functional unit but the elements become associated almost at random with the phage genetic material during the maturation of the phage particles prior to their release from the host cell (Streisinger and Franklin, 1956). This phenomenon is known as " phenotypic mixing ".

To test for a similar behaviour in T4D progeny of crosses between the $h_{111}$ and *tu45* mutants and wild type were compared with their parents for their ability to absorb to KS/4. The results for one such cross and its parental controls, *i.e.* $h41 \times h^+$ are given in fig. 2. The log adsorption curve of the progeny is clearly intermediate between that of its two parents both in the initial period of rapid adsorption and in the

level of adsorption at which further adsorption virtually ceases, namely, approximately 60 per cent. But what is more important both the *h41* and $h^+$ phage particles in the progeny while distinguishable by their plaque morphology on the indicator mixture, had identical rates of adsorption in spite of the enormous difference in rate of adsorption of the *h41* and $h^+$ parents. There is, therefore, phenotypic mixing for this character.

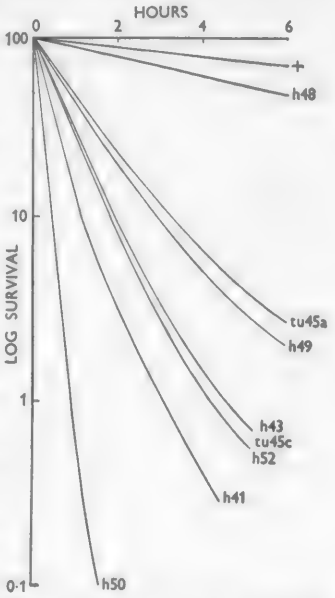


FIG. 3.—Graphs of the log percentage survival of mutants and wild type phage against time in hours at 45° C.

The technical simplicity of the heat inactivations plus the exponential form of the inactivation curves which would facilitate quantitative comparisons led to an investigation of phenotypic mixing for this character. The heat inactivation curves of the progeny from crosses between the heat sensitive $h_{111}$ and *tu45* mutants and wild type are given in fig. 4. Although parental controls were included in all these comparisons only the curve for wild type and the most and least sensitive mutants are included in the fig. 4 for the sake of clarity. In no cross is there a significant difference between the rate of inactivation of the mutant and wild type phage in the progeny, the relative frequency of the two genotypes remaining constant throughout the inactivations. Furthermore, the curves for the progeny are intermediate between those of their parents. We have, therefore, phenotypic mixing for heat sensitivity.

The quantitative aspects of the inactivation curves reveal two different situations. For *h41* and the *tu45* mutants the curves are essentially biphasic with an initial slope comparable with that of the mutant parent and a final slope with a back extrapolate around 70 per cent. approximating to wild type. The inactivation curves of the remaining progenies, that is those crossing *h43*, *h49*, *h50* and *h52* by wild type, are on the other hand triphasic. The initial slope is again like that of the mutant parent, similarly the final slope with a back extrapolate around 25 per cent. approximates to that of wild type. There is, however, a class of phage in the progenies with a sensitivity which is intermediate between that of its mutant and wild type parents. This latter fraction constitutes some 50 per cent. of the total having a back extrapolate around 70 per cent. thus coinciding with that of progenies from crossing *h41* and *tu45* to wild type.

### (vii) *The removal of phenotypic mixing*

A critical test of the phenotypic mixing explanation of the behaviour of the progeny of crosses between $h_{III}$ and *tu45* and wild type phage is provided by the results of recycling these progenies in a ten-fold excess of a bacterial host so that virtually all infections are by single phage particles. Under these conditions the mutant phage particles can only become associated with gene products of their own genes

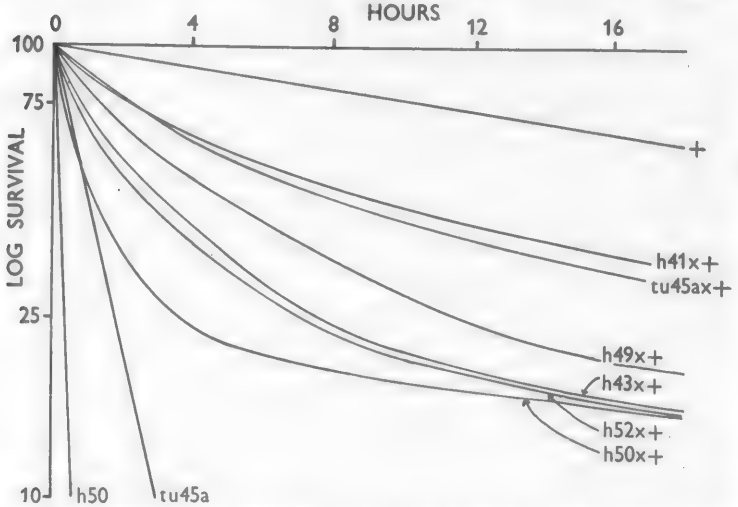


FIG. 4.—Graphs of the log percentage survival of the progenies of crosses of the $h_{III}$ and *tu45* mutants to wild type against time in hours at 45° C. In all cases the graphs for the mutants and wild types in the progenies are identical, hence there is phenotypic mixing for this character. The graphs for the wild type and the two extreme mutants used in these crosses are included for comparison. The mutants fall into two classes on the basis of these graphs. (1) Including $h_{41}$ and the *tu45* mutants are basically biphasic and (2) including all the remaining $h_{III}$ mutants are triphasic.

during maturation and similarly for the wild type phage. Hence, the mutant phage particles should emerge from the recycling with the heat sensitivity of the original mutant parent of the cross and the wild type phage with that of the original wild type parent. This is in fact the result obtained on recycling all the progenies described in the last section, thus supporting the explanation based on phenotypic mixing.

### (viii) *Functional tests within the* $h_{III}$-tu45 *region*

The heat sensitivity of the $h_{III}$ and *tu45* mutants and the phenotypic mixing for this character can be used to test whether the same or different functions are affected in the various mutants. Basically the qualitative test consists of making a mixed infection with a pair of mutants and examining the progeny for the existence of a heat-stable,

*i.e.* phenotypically wild type, fraction. The progeny of such mixed infections are of course already available from the crosses made to map the $h_{III}$ and *tu45* mutants.

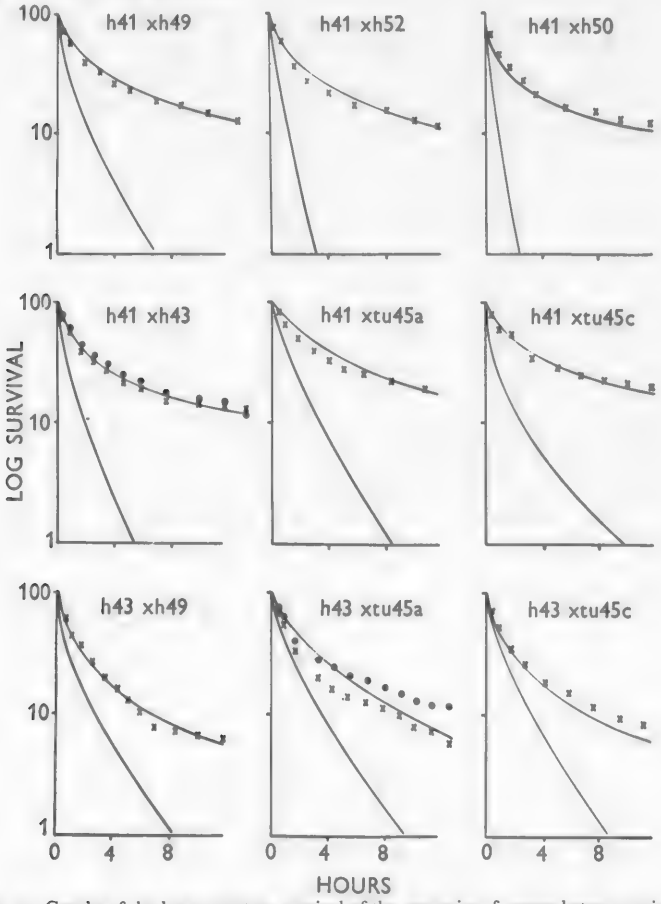


FIG. 5.—Graphs of the log percentage survival of the progenies of crosses between pairs of complementing mutants (X) of the $h_{III}$-*tu45* system against time in hours at 45° C. The upper curve of each graph is that expected if there is complementation and the lower curve if there is no complementation. For two of the crosses the progeny of the corresponding coupling cross is included (●) for comparison. These show that the *cis* and *trans* configurations give identical results if the mutants are functionally independent, *i.e.* if they complement.

An independent quantitative expectation for the size of the heat stable fraction can be obtained from the behaviour of the progenies from a mixed infection of each mutant with wild type during heat inactivation as described earlier and graphed in fig. 4. If we denote by $S_1(t)$ the fraction surviving as a function of time at 45° C. of the progeny from

a mixed infection of a mutant and wild type and by $S_3(t)$ the corresponding survival curve for a second mutant, then if independent functions are affected by the two mutants, the heat survival curve for

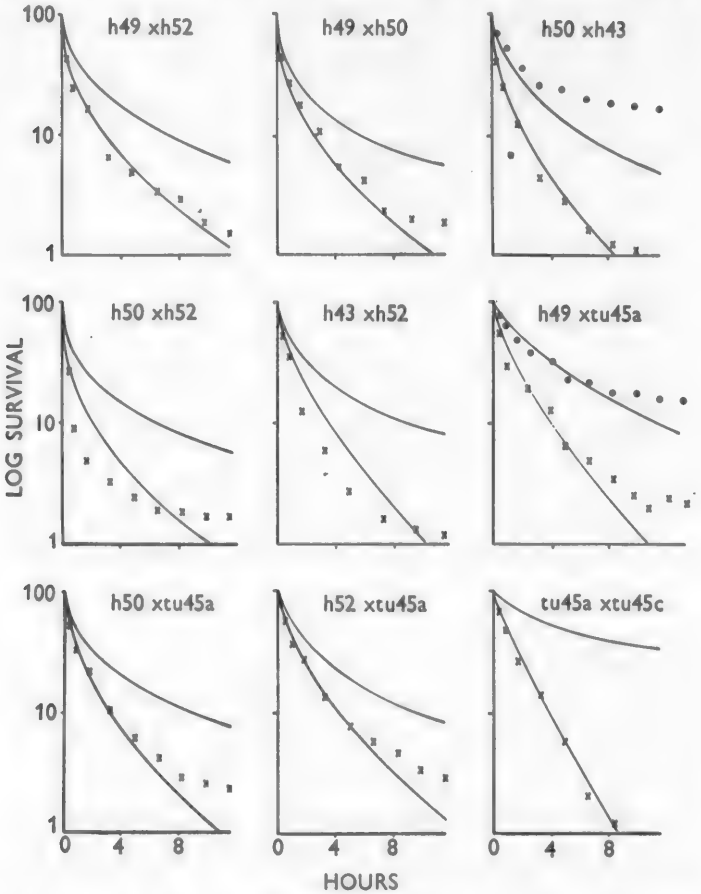


FIG. 6.—Graphs of the log percentage survival of the progenies of crosses between pairs of non-complementing mutants (X) of the *hIII-tu45* system against time in hours at 45° C. The upper curve in each graph is that expected if there is complementation and the lower curve if there is no complementation. For two of the crosses the corresponding coupling cross is included (●) for comparison. These show the lack of identity of the *cis* and *trans* configurations if the mutants affect the same function, *i.e.* if they do not complement.

the progeny from a mixed infection of the two mutants should be simply the product $S_1(t) \times S_2(t)$. If the mutants affect the same function and do not interact the survival of the progeny should be the same as the parents given in fig. 3.

For each pair of mutants, therefore, there are two expectations for the rate of inactivation of their progeny from a mixed infection, one assuming that they affect the same function and the other that they affect different functions. In figs. 5 and 6 the inactivation curves

TABLE 1

*Classification of the functional relationships of the* $h_{III}$ *and* tu45 *mutants based on the inactivation curves in figs. 5 and 6. A plus indicates functional independence of the two mutants and a minus functional dependence,* i.e. *complementing and non-complementing pairs respectively.*

| | *h41* | *h49* | *h52* | *h50* | *h43* | *tu45a* | *tu45c* |
|---|---|---|---|---|---|---|---|
| *h41* | | + | + | + | + | + | + |
| *h49* | | | − | − | + | − | − |
| *h52* | | | | − | − | − | − |
| *h50* | | | | | − | − | − |
| *h43* | | | | | | + | + |
| *tu45a* | | | | | | | − |
| *tu45c* | | | | | | | |

observed are compared with these two expectations individually for each pair of mutants. The results are quite unambiguous, the inactivation curves for the progenies following one or other of the expected curves for every cross. The qualitative conclusions from these graphs are given in table 1, where a plus indicates functional independence between the two mutants.

*h41* and its alleles (*h44*, *h45*, *h46* and *h47*) affect a different function, or belong to a different cistron (Benzer, 1955) from all the other heat

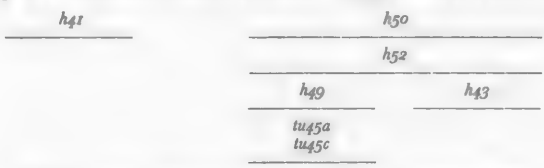


FIG. 7.—Linear complementation map of the *hIII*-*tu45* mutants.

sensitive mutants. The remaining mutants, however, do not constitute a single functional group or cistron and mutant *h43* appears to affect a different function from *h49* and the *tu45* mutants although like the latter group it does not appear to affect a different function from *h50* and *h52*. Clearly no unambiguous subdivision of this group of mutants into independent functional units is possible. It is, however, possible to draw a linear " complementation map " of the type used in presenting similar data from enzymatic mutants of *Neurospora* (see Fincham, 1959; Beadle, 1960; Catcheside, 1960, for review) but such a complementation map (fig. 7) is not colinear with the genetic map (fig. 1).

The data from the heat inactivations of the progenies of pairs of mutants also provide additional evidence of phenotypic mixing for heat sensitivity. Thus in the progenies of two $h_{III}$ or *tu45* mutants there is

always a small fraction of wild type recombinants and in the progenies of an *h*III and a *tu45* mutant all four phenotypes in the progeny can be classified. It is a simple operation therefore to confirm that all the genotypes in a single progeny have identical heat sensitivity. Similarly it is easily shown that following a single recycling of these progenies in a ten fold excess of bacterial host cells the phage particles re-emerge with the heat sensitive characteristic of their individual genotypes.

### (ix) *Cis-trans comparisons*

Another test of the functional independence of pairs of mutants is provided by comparisons of the heat inactivation curves of progeny from mixed infections with the same pair of mutants in the coupling (or cis) and repulsion (or trans) configurations. For mutants involving different functions, the curves should be essentially identical, otherwise the survival should be much greater with the coupling configuration. Indeed in the latter case the coupling cross might be expected to behave like that between a single mutant and wild type (*e.g.* fig. 4).

As a preliminary to these comparisons, it was necessary to isolate the coupling combinations of pairs of the *h*III and *tu45* mutants. This had already been done on a limited scale to establish the *h* phenotype of the double *h* mutants which arose as recombinants during the mapping experiments. To obviate the need for some hundred backcrosses to isolate a single coupling combination a selective technique was sought. Stocks of *h*III-*tu45* mutants in coupling were readily obtained as recombinants from the corresponding repulsion crosses on the basis of their morphology on the mixed indicator. Stocks of these double mutants had heat sensitivities which were the product of those of their component single mutants. On the assumption that this relationship would extend to stock of pairs of *h*III mutants in coupling the phenotypically *h* plaques from the progeny of repulsion crosses between pairs of *h*III mutants were scored for heat sensitivity. One hundred *h* plaques from each such cross were isolated into E.H.A. broth and assayed before and after heat inactivation for one hour at 45° C. From each cross four or five of the isolates had survival rates at the end of the inactivation which were much lower than the remainder. These were built into stocks in the usual way and backcrossed to both parents to confirm their double mutant nature. In every case the isolates selected on their greater heat sensitivity proved to be double mutants on the backcross test. Indeed it was subsequently shown, in properly controlled experiments, that the double *h*III mutant stocks had heat sensitivities which were exactly the product of the heat sensitivities of their component single mutants.

The double mutant stocks *h41h43*, *h43tu45a*, *h43h50* and *h49tu45a* were all crossed to wild type and the heat inactivation curves of their progenies from the mixed infections compared with those from the corresponding repulsion crosses. In every case our earlier conclusions concerning the functional relationships of the *h*III and *tu45* mutants

(figs. 5 and 6, table 1) and the expectations derived from them were confirmed.

In principle the methods used in determining the functional relationships between the $h_{111}$ and *tu45* mutants are directly applicable to the detection of " partial complementation " of the type which is now well known in *Neurospora* (Fincham, 1959; Catcheside, 1960; and Beadle, 1960). That is, the production of a proportion of heat-stable phage progeny by a pair of mutants in mixed infection which is less than that expected on the basis of complete functional independence of the mutants. And indeed some of the apparently non-complementing pairs of mutants, *e.g.* *h49*×*h50*, *h50*×*h52* and *h49*, *h50* and *h52*×*tu45a* in fig. 6 and table 1 do in fact appear to give a small proportion of heat-stable phage progeny. But the heat inactivation curves for these combinations do not differ significantly from the expectations based on the functional identity of the pairs of mutants involved. Furthermore, an alternative source of a small heat-stable fraction in the progenies would need to be eliminated before considering seriously an explanation based on partial complementation. This alternative source is the possibility of a small contribution of heat-stable elements to the pool within the mixedly infected bacterial cells by the small percentage of wild type recombinant phage particles produced by all the pairs of mutants involved in these functional tests. Clearly, until the magnitude of any such contribution by the wild type recombinants is known speculation on the existence of partial complementation is fruitless.

A peculiarity shown solely by the heat inactivations of the progenies of the coupling crosses is the slight, but significant, association between genotype and phenotype for heat sensitivity so that the wild type in a progeny are more heat-stable than the mutants in the same progeny, *i.e.* " phenotypic mixing " is incomplete. As a consequence the proportion of wild types in the surviving progeny increases during the course of the heat inactivations. While offering no rigorous proof of the origin of this peculiarity it could arise from the small proportion of bacterial host cells which are infected by only one parent during the crosses which produce the progenies used in the heat inactivations. These will inevitably lead to the production of a corresponding proportion of the progeny in which genotype and phenotype will be completely correlated for heat sensitivity. This correlation would remain undetected in the repulsion crosses where both parents have approximately the same heat sensitivity. When, however, the parents differ so enormously in heat sensitivity as do the double mutants and wild type in the coupling crosses even a small amount of correlation is sufficient to cause detectable disturbances.

### (x) *The number of elements controlling heat sensitivity*

It is quite clear that in phage T4D as in phage T2*h* the elements of the phage particle responsible for their heat stability are produced in

a pool. And while the specificity of the elements is determined by the *h*$_{III}$ and *tu45* functional units in T4D the elements become associated more or less at random with phage genetic material during the course of maturation. In T2*h* it has been shown that at least two elements are associated with each phage particle, but the number may be larger (Streisinger, 1957). The determination of this number is based on a method of Hershey and depends on the agreement between the proportion of heat stable phage emerging from a mixed infection of different multiplicities of a sensitive and stable parent and the proportion expected on the assumption of 1, 2 or more elements per phage.

Only one multiplicity has been used in the experiments with phage T4D but similar arguments may be used to arrive at an estimate of the minimal number of elements per phage.

The mixed infections between the heat sensitive mutants and the heat stable wild type gave progenies which fell into two classes (1) giving 65 to 75 per cent. and (2) giving 25 to 30 per cent. heat stable, *i.e.* phenotypically wild type, progeny (fig. 4). In both classes only about 25 per cent. of the phage progeny had the sensitivity of the mutant parent, the balance of around 50 per cent. in those of class (2) being intermediate in sensitivity. The mere existence of the intermediates with hybrid phenotypes proves that phage particles can acquire elements from both the mutant and wild type parental contributions to the pool within the mixedly infected host cells. There must, therefore, be more than one element per phage and the following considerations suggest that there are probably two. Thus if it is assumed that there are two elements per phage and further that the mutant and wild type phage particles in mixedly infected cells contribute elements equally to the pool from which the progeny phage draw at random during maturation, then, 25 per cent. will draw both elements from the wild type contribution, 25 per cent. both elements from the mutant contribution and 50 per cent. one element from each. So far the model makes no distinction between class (1) and (2) behaviour. To explain this class difference a further assumption is required, namely, that while only one wild type element per phage is required to give the wild type heat stability for progenies which belong to class (1) both elements must be wild type to achieve this stability in progenies of class (2).

In so far as the form of the heat inactivation curves of the complementing pairs of mutants are predictable from those of the individual mutants crossed to wild type (figs. 4, 5 and 6) they can provide no independent evidence for or against the estimate of two elements per phage.

## 4. DISCUSSION

All the *h*$_{III}$ and *tu45* mutants used in this investigation give recombinants on crossing, there is, therefore, no structural overlap between them. There is, however, functional overlapping although every one of the mutants will complement at least one other. The pattern

of complementation is not simple. One mutant *h41* and its alleles is clearly a distinct functional group or cistron in that it complements all other known mutants in the $h_{III}$-*tu45* system. No unambiguous classification of the functional relationships of the remaining mutants in the system is, however, possible, hence no subdivisions into cistrons can be made. All the complementation on which the classification of functional relationships has been attempted is equally efficient, that is, none is " partial " in the sense used in *Neurospora* (see Fincham, 1959 ; Catcheside, 1960; and Beadle, 1960, for reviews) although the existence of " partial complementation " between pairs of mutants classified as non-complementing cannot be ruled out on the evidence presented here. From the functional relationships a linear complementation map can be constructed (fig. 7) but this has no correspondence with the unambiguous linear order of the mutants established by recombination studies. Thus the simplicity of the relationship between gene function and structure in the $r_{II}$ region of phage T4 which led to the formulation of the cistron concept in phage (Benzer, 1955) is not repeated in the $h_{III}$-*tu45* system.

Since internal suppressors of the heat sensitivity of the $h_{III}$-*tu45* system which map within the *III* region throw further light on the organisation of this chromosome segment further discussion will be held over until these results are presented in a later paper (Jinks, 1961).

## 5. SUMMARY

Wild type phage T4D adsorbs very poorly to bacterial strain KS/4 and gives turbid ghost plaques on a mixed indicator of strains KS/4 and S/6. It is possible to isolate phage mutants designated *h*, which adsorb more readily to KS/4 and give clear plaques on the mixed indicator. Most of these mutants are located within a region of about 12 map units on the third linkage group between *m41* and *tu45*.

Mapping experiments have allowed ten of these mutants to be unambiguously ordered relative to each other and to *tu45*.

At 45° C. the $h_{III}$ and *tu45* mutants are exponentially inactivated at rates varying from 2 to 100 times that of wild type. This property can be used to test whether the same or different functions are affected in the various mutants.

Qualitatively the test consists of determining whether or not a pair of mutants can produce heat-stable phage particles when mixedly infected into bacterial host cells. A quantitative test allows the proportion of heat stable phage produced to be compared with an expectation based on the proportion of heat stable phage which each mutant of a complementing pair produces when mixedly infected with wild type phage. Further evidence in some instances is provided by *cis* and *trans* comparisons.

All agree in the classification into complementing and non-complementing pairs. The results obtained, however, do not allow the mutants to be grouped into functional units or cistrons. It is possible

to draw a linear complementation map which is not colinear with the genetic map.

*Acknowledgments.*—This work was carried out during the tenure of a Harkness Fellowship of the Commonwealth Fund.

I am indebted to Dr R. S. Edgar who suggested the problem and who along with Mr C. M. Steinberg and Dr R. H. Epstein provided much helpful discussion.

This work was financed in part by the National Foundation.

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# A FURTHER STUDY OF FITNESS OF CHROMOSOMALLY POLYMORPHIC AND MONOMORPHIC POPULATIONS OF *DROSOPHILA PSEUDOOBSCURA* *

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Received 14.ii.61

## 1. INTRODUCTION

THE present report is a sequel to that of Beardmore, Dobzhansky and Pavlovsky (1960), who made an exploratory study of the fitness of chromosomally polymorphic and monomorphic experimental populations of *Drosophila pseudoobscura*. The populations of this previous study were kept for about 3 years in wood-and-glass cages, each with 15 cups containing Drosophila culture medium. A cup of fresh medium was introduced, and one with used-up medium removed, on alternate days. The populations each contained some 1000-4000 adult flies, and at least ten times as many eggs and larvæ. The polymorphic populations had flies with AR and with CH gene arrangements in their third chromosomes; the homokaryotypes AR/AR and CH/CH, and the heterokaryotype AR/CH, were represented with frequencies approaching those demanded by the Hardy-Weinberg rule. The monomorphic populations had only AR, or only CH, chromosomes. The study has shown that the polymorphic populations produced more flies per cup of culture medium than did the monomorphic ones. Individual flies in the polymorphic populations were neither consistently larger nor heavier than in the monomorphic ones; however, since the former produced more flies, they produced also a greater fly biomass. Furthermore, the variances of the numbers of the flies produced per cup, and the variances of individual weights and of sizes were lower in the polymorphic than in the monomorphic populations. The conclusion seems warranted that, under the conditions of the experiments, the polymorphic populations exploit more efficiently the resources of their environment than do the monomorphic populations.

The environment offered to Drosophila in the experimental population cages is a highly competitive one. The food given is sufficient to sustain the development to the adult stage of fewer than 10 per cent, though probably of more than 1 per cent, of the eggs deposited. However, the adult flies probably do not starve; a part of the surface of the yeasted food medium is almost always free of feeding or ovipositing flies. In the experiments to be described below the situation is different. In these experiments we have used a variant

* The work under Contract No. AT-(30-1)-1151, U.S. Atomic Energy Commission.

of the technique first proposed by Buzzati-Traverso (1955), and developed by Carson (1958) and his collaborators. Here the adult flies are crowded in a space so confined that they interfere with each other, probably largely mechanically. After an initial spurt of oviposition, the number of the eggs deposited in the populations drops, and many eggs are probably trampled by the adults and do not hatch. The result is that the population sizes become limited, chiefly owing to the competition among the adults rather than among the larvæ. We hope to show that under these conditions the polymorphic populations are superior in fitness to the monomorphic ones.

## 2. EXPERIMENTAL PROCEDURE

The material for the present experiment was derived from the same experimental populations which were utilised by Beardmore, Dobzhansky and Pavlovsky (1960). In December of 1958 and in January of 1959, fifty pairs of adult flies were taken from each of the populations Nos. 173 and 181 (polymorphic), 175 and 180 (monomorphic for AR), and 177 and 182 (monomorphic for CH). Each group of fifty "founders" were placed in culture bottles with fresh food. Twice a week (on Mondays and Thursdays, or on Tuesdays and Fridays), the adult flies were transferred, without etherisation, to fresh culture bottles. The eggs which they deposited were allowed to develop and to yield adults. When the hatching of the progeny began, the bottles were emptied twice a week, the flies etherised, females and males separated, weighed and added to the adult population. Once a week, the adult population was etherised, females and males separated, counted, weighed and placed in a bottle with fresh food. The adult ovipositing flies were thus always in a single bottle with fresh food, while 7-8 bottles in each series contained eggs, larvæ, pupæ and newly hatched adults. All the bottles were kept in incubators at 25° C. The weighings were made on a chemical balance, with a precision of 0·1 mg.

The number of flies in the experimental populations underwent characteristic changes, very much the same in all cases. The course of events in the populations Nos. 180, 181 and 182 is represented graphically in fig. 1. Being relatively uncrowded, the 50 pairs of "founders" of each population oviposit freely. In about 4 weeks from the start, the numbers of the adult flies begin to grow rapidly, owing to the ecclosion of young flies. By the 10th-12th week the adult population increases to 1000 or more flies. This causes a fearful overcrowding. The 3-day intervals between the transfers of the flies to fresh cultures suffice to have the whole surface of the nutrient medium in the bottles covered by cadavers of the flies which have died during the interval. The numbers of progeny hatching from the bottles in which the parents were so overcrowded become small, apparently because relatively few eggs are laid, and many of them

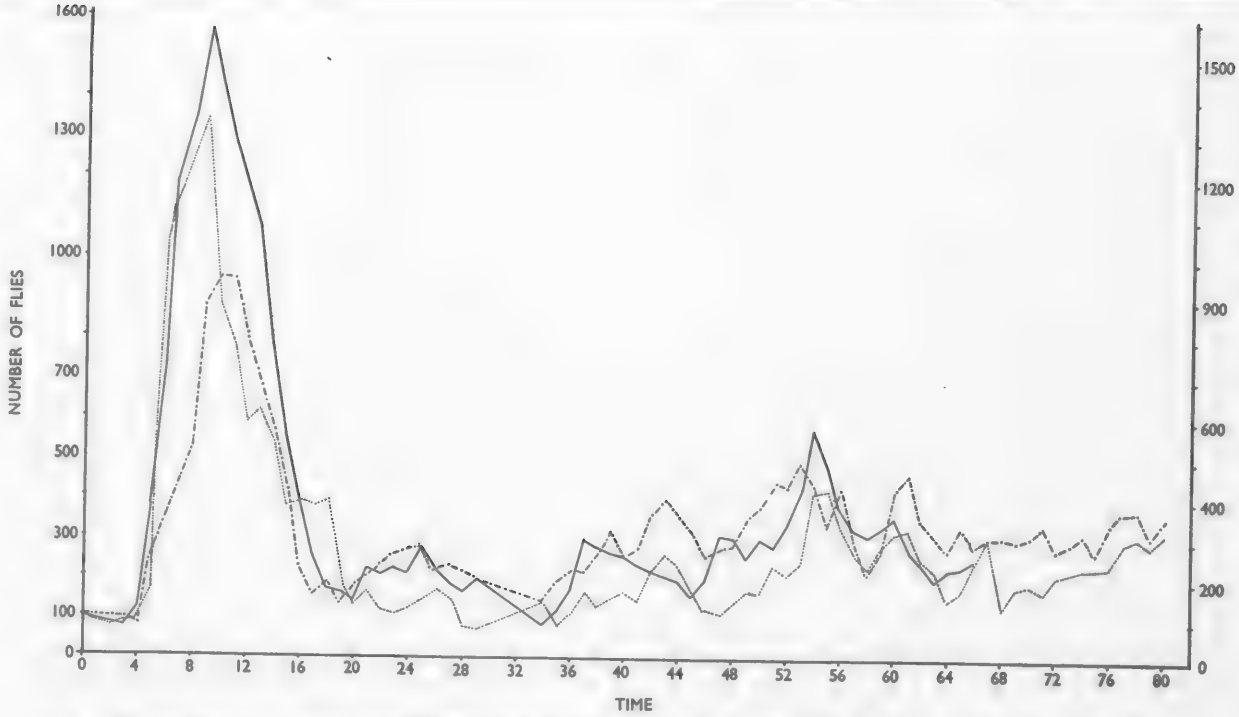


FIG. 1.—Numbers of adult flies in the monomorphic and polymorphic populations. Abscissa—time in weeks ; ordinates—numbers of flies. Solid line—the monomorphic CH population (No. 182) ; dotted line—the monomorphic AR population (No. 180) ; dot-and-dash line—the polymorphic population (No. 181).

fail to develop. By about the 15th-18th week the adult population is reduced back to about 100-300 flies.

The diminished crowding of the adults permits more eggs to be laid and to develop. From then on, the populations undergo rather irregular oscillations, the character of which can be seen in fig. 1 more easily than it can be described in words. The experiments were continued for 70 to 85 weeks. Towards the middle of this period, a trend became apparent towards a gradual increase in the population size, as can be seen in fig. 1. Whether this trend was due to a progressing genetic adaptation to the experimental conditions, or to the improving art of the experimenter is uncertain; the former is more likely to be the correct explanation than the latter.

Let us now ignore the first 18 weeks of the existence of the populations, during which the populations first "explode" and then "crash". From the 18th week on, the oscillations do not, at first sight, show a clear advantage of any one population (fig. 1). A more careful inspection discloses, however, that the polymorphic population (No. 181) had often most flies, while the monomorphic AR population (No. 180) had normally fewest, the monomorphic CH (No. 182) being intermediate. This visual impression can be tested statistically.

Tables 1 and 2 show the means and their standard errors for the following parameters: the numbers of flies of either sex in the adult populations and their weights (as determined from the weekly counts and weighings); the numbers of flies of either sex hatched from the pupæ per half-week, and their weights; the tables also show the mean weights of a female and of a male fly in the populations; these means are obtained simply by division of the total weight by the number of individuals of a given sex. Because of the trend towards a gradual increase in the population sizes (see above), we have computed the means only for the populations which were studied strictly simultaneously. As shown in fig. 1, population No. 182 had to be destroyed 14 weeks before Nos. 181 and 180. Therefore the data shown in table 2 take into consideration only the time interval when all these populations were more than 18 weeks but less than 66 weeks old.

### 3. THE FIRST EXPERIMENT

The three populations, No. 173 (polymorphic), No. 175 (monomorphic AR), and No. 177 (monomorphic CH) had similar histories while they lived in the population cages, and the bottle populations derived from them were studied simultaneously. Table 1 reports the sizes of the adult populations and the birth rates of the adults in these populations. The means are calculated for 48 weeks (18th-70th; no counts were taken from the 25th to the 29th week).

The polymorphic population contained on the average more adult flies, both females and males, than the monomorphic CH population, and the latter more than the monomorphic AR population. However,

while the differences between the polymorphic and the monomorphic CH are not statistically significant, those between the monomorphic CH and the monomorphic AR are statistically assured. The same sequence, polymorphic >monomorphic CH >monomorphic AR, is observed also for the total weights of the adult females and of males, the numbers of females and of males born (*i.e.* hatched from pupæ) per unit time, and the weights of females and males born per unit time (table 1). The differences between the polymorphic and the

TABLE 1

*Number and weight (in mg.) of the flies in the populations. First experiment*

| | | Polymorphic AR/CH(173) | Monomorphic AR(175) | Monomorphic CH(177) |
|---|---|---|---|---|
| Total population | ♀♀ | 217·5±7·1 | 155·4±4·3 | 216·8±5·1 |
| | ♂♂ | 139·9±6·0 | 106·0±4·4 | 132·0±4·3 |
| Total weight | ♀♀ | 265·2±9·2 | 192·9±5·1 | 247·3±6·1 |
| | ♂♂ | 124·3±6·1 | 92·3±3·9 | 110·9±3·7 |
| Mean weight | ♀ | 1·22±0·06 | 1·24±0·04 | 1·14±0·05 |
| | ♂ | 0·89±0·04 | 0·87±0·05 | 0·84±0·04 |
| Born per 3½ days | ♀♀ | 69·9±2·4 | 51·6±1·8 | 66·9±1·7 |
| | ♂♂ | 45·8±1·7 | 36·6±1·3 | 42·4±1·3 |
| Weight per 3½ days | ♀♀ | 80·8±2·9 | 59·0±2·2 | 72·0±2·0 |
| | ♂♂ | 39·1±1·6 | 29·8±1·2 | 33·7±1·2 |
| Mean weight, newborn | ♀ | 1·16±0·06 | 1·14±0·05 | 1·08±0·04 |
| | ♂ | 0·85±0·05 | 0·81±0·04 | 0·79±0·04 |

monomorphic CH populations mostly fall short of statistical significance, while those between the monomorphic CH and AR populations are quite significant, and so are the differences between the polymorphic and the monomorphic AR populations.

Table 1 shows also the mean weights of the newly hatched females and males, and of the females and males in the adult ovipositing populations. These data are suggestive in several respects. A newly-hatched female is found to weigh about 1·1 mg., and a newly-hatched male about 0·8 mg. In the experiments of Beardmore, Dobzhansky and Pavlovsky (1960, tables 3, 4 and 5), females weighed 0·8-0·9 mg., and males 0·7-0·8 mg. These figures are not strictly comparable, since in the experiments of Beardmore *et al.*, the flies were weighed at an average age of less than one day, and in the present experiments they were weighed at an average age of about 1·5 days. The figures, nevertheless, suggest that in the present experiments the flies were larger, and this is in accord with our visual impression. This is as it should be, since the larvæ in the bottle populations are much less crowded and have more abundant food than they do in the cage populations. On the other hand, the flies in the adult ovipositing

populations were only slightly heavier than were the freshly hatched flies (about 1·2 mg. instead of 1.1 mg. per female, less than 0·9 mg. instead of 0·8 mg. per male, see table 1). The crowding of the adults in a single bottle evidently prevented them from securing enough food and from gaining much weight.

## 4. THE SECOND EXPERIMENT

The polymorphic population No. 181, and the monomorphic AR (No. 180), and CH (No. 182) populations, were established originally from the same source as Nos. 173, 175 and 177. However, the " 180-series " spent some months at a lower temperature (16° C.), and were

TABLE 2

*Number and weight (in mg.) of the flies in the populations. Second experiment*

| | | Polymorphic AR/CH(181) | Monomorphic AR(180) | Monomorphic CH(182) |
|---|---|---|---|---|
| Total population | ♀♀ | 184·3±5·6 | 139·1±6·1 | 169·2±7·5 |
| | ♂♂ | 115·5±5·5 | 74·3±4·5 | 101·8±5·9 |
| Total weight | ♀♀ | 221·9±6·1 | 168·3±6·9 | 204·1±9·3 |
| | ♂♂ | 103·4±5·2 | 62·8±3·9 | 89·3±5·8 |
| Mean weight | ♀ | 1·20±0·05 | 1·21±0·06 | 1·21±0·06 |
| | ♂ | 0·90±0·05 | 0·85±0·09 | 0·88±0·07 |
| Born per 3½ days | ♀♀ | 59·3±2·0 | 46·1±2·0 | 52·0±2·0 |
| | ♂♂ | 38·2±1·6 | 26·2±1·4 | 32·2±1·7 |
| Weight per 3½ days | ♀♀ | 66·4±2·2 | 48·7±2·2 | 59·6±2·4 |
| | ♂♂ | 32·9±1·4 | 20·9±1·1 | 28·1±1·6 |
| Mean weight, newborn | ♀ | 1·12±0·05 | 1·06±0·06 | 1·15±0·06 |
| | ♂ | 0·86±0·05 | 0·80±0·06 | 0·87±0·07 |

then returned to 25° C. The two " series " of populations behaved somewhat differently (*cf.* Beardmore, Dobzhansky and Pavlovsky, 1960). As shown in table 2, the " 180-series " contained and produced consistently somewhat fewer flies than the " 170-series " (table 1). However, the three populations of each series are quite comparable.

Table 2 shows that the polymorphic population (No. 181) contained more flies of both sexes, and produced more flies per unit time, than the monomorphic CH (No. 182), and this latter contained and produced more flies than the monomorphic AR (No. 180). The differences between Nos. 181 and 182 are quite consistent, although some of them do not reach the conventional level of statistical significance. The differences between Nos. 182 and 180, and *a fortiori* between Nos. 181 and 182, are significant. On the other hand, the mean weights of a fly are about the same in all populations.

The results of the first and the second experiments (the two " series " of the populations) are mutually consistent.

## 5. LONGEVITY

In a population at equilibrium, the mean numbers of births and of deaths per unit time are equal. The mean numbers of the flies in the populations, and the mean numbers of the flies born per unit time being known, the mean longevity of the flies in the experimental populations can be calculated. The calculation raises some statistical problems, which Professor Howard Levene has kindly consented to discuss in the Appendix to the present paper. The estimates are reported in table 3.

The longevity of the females is slightly but consistently greater than that of the males. The longevity of either sex is greatest in the monomorphic CH populations, intermediate in the polymorphic, and lowest

TABLE 3

*Estimated mean longevity, in days, of the adult flies in the population*

| Population | Females | Males |
|---|---|---|
| No. 173, Polymorphic . . | 12·05 | 11·85 |
| No. 175, Monomorphic AR . | 11·70 | 11·30 |
| No. 177, Monomorphic CH . | 12·50 | 12·06 |
| No. 181, Polymorphic . . | 12·04 | 11·74 |
| No. 180, Monomorphic AR . | 11·72 | 11·09 |
| No. 182, Monomorphic CH . | 12·55 | 12·22 |

in the monomorphic AR populations. All the longevities in our experimental populations are much lower than in flies of the same species and at the same temperature but under near-optimal conditions (*cf.* Vetukhiv, 1957).

## 6. DISCUSSION

In the present experiments, like in those of Beardmore, Dobzhansky and Pavlovsky (1960), chromosomally polymorphic populations evince a higher fitness than do the chromosomally monomorphic ones. The polymorphic populations contain more individuals, and a greater biomass, than the monomorphics. The polymorphic populations utilise their environments more efficiently. The mean size and the mean longevity of an individual are, however, not very different in the polymorphic and monomorphic populations.

As pointed out in the Introduction, the environments which the populations were called upon to master were different in the experiments of Beardmore, Dobzhansky and Pavlovsky and in the present experiments. In the former, the larvæ were exposed to extreme crowding, while the adults were relatively uncrowded, while in the present ones the conditions are reversed. This environmental difference is reflected in the results. While in the former experiments the monomorphic CH populations were inferior to the monomorphic AR, in

the present ones CH is clearly superior to AR, and, in point of fact, not much below the polymorphic populations.

The reversal of the relative adaptive values of the monomorphic CH and AR populations is, we believe, quite significant ecologically. In the populations of the localities in California from which the wild progenitors of our experimental flies came, the relative frequencies of chromosomes with the CH gene arrangement increase during the spring but decrease during the summer (Dobzhansky, 1947). Another class of chromosomes, ST, wane during the spring and wax during the summer. The AR chromosomes are relatively stable in frequency. Now, in the experimental populations kept in population cages the homozygous ST/ST class is more fit than AR/AR, and the latter is fitter than CH/CH (at 25° C.), whereas the fitnesses of all of them are approximately equal at 16° C. (Dobzhansky, 1948). Yet under natural conditions in spring, CH/CH must be more fit than AR/AR and ST/ST, since the frequency of the CH class is on the increase. The experiments of Birch (1955) have been the first to shed needed light on this situation. Birch showed that when the crowding of the larvæ is eliminated, the CH/CH karyotype is superior in fitness to ST/ST. Our results may, then, be regarded as a confirmation and extension of those of Birch. In the population cages the competition among larvæ for food is severe, while in our "bottle populations" the competition is transferred to the adult stage. The inversion of the fitness order from AR/AR >CH/CH in the experiments of Beardmore, Dobzhansky and Pavlovsky (1960) to CH/CH >AR/AR in the present experiments is thus explained.

## 7. SUMMARY

Experimental populations of *Drosophila pseudoobscura* were made polymorphic and monomorphic for the AR and CH gene arrangements in their third chromosomes. In contrast to the previous experiments of Beardmore, Dobzhansky and Pavlovsky (1960), in which the larvæ were crowded and the adult flies relatively uncrowded, in the present experiments the adults were crowded and the larvæ relatively uncrowded. The chromosomally polymorphic populations proved to be superior to the chromosomally monomorphic ones; the former produced more flies and a greater biomass, although the average weights of the individual flies were about alike. In the experiments of Beardmore, Dobzhansky and Pavlovsky (*loc. cit.*) the monomorphic AR/AR were superior to the monomorphic CH/CH populations. In the present experiments this order is reversed. The bearing of this reversal on the seasonal changes in the genetic constitution which the populations undergo in their natural habitats is pointed out.

*Acknowledgments.*—We are obligated to Professor H. L. Carson, of the Washington University, St Louis, for advice concerning the experimental technique, to Professors Howard Levene of Columbia University and L. C. Birch of the University of Sydney for useful discussions, and to Mrs. Angela Drescher for help with the calculations.

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## APPENDIX

HOWARD LEVENE

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*The estimation of longevity.* If the birth process and death process are both continuous stationary stochastic processes of an arbitrary kind, the mean longevity is equal to the mean number of living individuals, $N$, divided by the mean number born per unit interval of time, $B$. In the present case the death process is approximately continuous : that is individuals die one by one at random times. However, the birth process is essentially discrete. For the present purpose " birth " is not the time of biological birth, but the time at which an individual enters the population. Individuals enter the population in batches, alternately all flies born and surviving in a 3-day period and all flies born and surviving in a 4-day period. Since " births " are only counted on addition of flies, for mathematical purposes flies are born when they are added, and deaths only begin at that time. If mean longevity were long, compared with the interval between births, this would matter little, but in the present experiment the mean longevity is only about three times the mean interval between births. Accordingly a correction factor must be introduced.

The correction factor will depend on the distribution of time of death. We will use the simplest such distribution, the exponential, where the probability an individual born at time zero dies by time $t$ is $1-e^{-t/m}$, where $m$ is the mean longevity expressed in the same units as $t$. This distribution arises if the probability a fly alive at time $t$ dies between $t$ and $t+\Delta t$ is $\Delta t/m$ regardless of the age of the fly. Since in the present experiment most flies die well before old age as a result of overcrowding, in large part from purely accidental causes, this model may not be too far in error.

If flies had been added at uniform time intervals $c$, the expected number of flies present at the beginning of the interval that die during it is $(N+B)(1-e^{-c/m})$, and the proportion remaining alive would be $N=(N+B)e^{-c/m}$,

where $(N+B)$ is the number alive at the beginning of the period. When a stable population size is reached, the number of individuals " born " at the beginning of each period must be $B = (N+B)(1-e^{-c/m})$, giving

$$(N+B)e^{-c/m}+(N+B)(1-e^{-c/m}) = (N+B)$$

at the start of the next period.

We then have the crude measure of mean longevity in terms of the time interval $c$ as our unit,

$$\frac{a}{c} = \frac{N}{B} = \frac{Ne^{-c/m}}{N(1-e^{-c/m})} = \frac{e^{-c/m}}{1-e^{-c/m}} \neq \frac{m}{c}, \tag{1}$$

where $m$ is the true mean longevity and $a$ the crude measure, both in the original units of time. The closeness of $a$ to $m$ depends on the ratio $r = m/c$, being closer the larger the mean longevity is relative to the intervals $c$. This can be seen by evaluating the expression (1) numerically ; however, by expanding the exponentials in (1) in power series, simplifying, and dividing algebraically, it can be shown that

$$r = \frac{m}{c} = \frac{a}{c} + \frac{1}{2} - \frac{1}{12r} + \frac{1}{24r^2} - \quad \cdots \quad . \tag{2}$$

Furthermore the error committed by stopping with any number of terms on the right side of this expression is less than the first term omitted.

In the present situation we have noted that $r$ is approximately 3, and we have

$$\frac{m}{c} - \frac{a}{c} = \frac{1}{2} - \frac{1}{(12)(3)} + \frac{1}{(24)(3)^2} = 0{\cdot}5-0{\cdot}02778+0{\cdot}00463,$$

so that the first correction term, 0·5, is important, but the others are not.

The actual situation in the present experiments is more complicated, since two different time intervals, $c = 3$ days and $d = 4$ days are involved in alternation. Counting of survivors was always done before addition of " new-born " flies after the *shorter* period (*e.g.* if flies were added on Monday and Thursday, counting was done on Thursday before the addition of new flies). Now we must let $b$ = the mean number of flies born *per day*, so that the number born in a period of $c$ days is $cb$ and the number born in a period of $d$ days is $db$. Then at equilibrium we must have the relationship

$$N = [(N+cb)e^{-d/m}+db]e^{-c/m}. \tag{3}$$

Since the crude measure of longevity is $a = N/b$ in days, we have $N = ab$, and substituting in (3) we have

$$ab = [(a+c)be^{-d/m}+db]e^{-c/m}. \tag{4}$$

Dividing both sides by $b$, $b$ no longer appears, and solving for $a$ gives

$$a = \frac{ce^{-\frac{c+d}{m}}+de^{-\frac{c}{m}}}{1-e^{-\frac{c+d}{m}}} = \frac{3e^{-7/m}+4e^{-3/m}}{1-e^{-7/m}} \tag{5}$$

for our special case. Setting $c = d$, and $e^{-2c/m} = (e^{-c/m})^2$ it can be shown that the first expression in (5) reduces to $c$ times the expression (1) as it

should. It is easily shown algebraically or numerically that for this case also $(m-a)$ is nearly constant for small variations in $m$. Thus for $m = 11$, $m-a = 1{\cdot}1591$, while for $m = 12{\cdot}6$, $m-a = 1{\cdot}1656$.

In the present study, the crude longevity is obtained by dividing the mean number of flies present by the mean number born per 3·5 days and multiplying by 3·5 in tables 1 and 2, giving crude $a$'s. For the range of $a$ values found, this can be corrected to estimate $m$ with two decimal accuracy, by simply adding 1·16, and this has been done in table 3.

It should be noted that the corrections are based on a death rate independent of age. If the death rate increases with age the correction required will be smaller. If the death rate is high at first, then low, and finally increases, the correction will be closer to that for constant rate. Since it is reasonable to assume a fairly constant death rate in this experiment, the corresponding correction has been used.

It may also be noted that these longevities are measured from the time the flies are placed in the crowded cultures. On the average, flies are about 1·75 days old when added. There is also some early unrecorded mortality in the bottles where the flies are born.

# CHROMOSOME NUMBER AND BEHAVIOUR IN THE GRASSHOPPER *PHOLIDOPTERA*

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Received 6.iii.61

## 1. INTRODUCTION

CHROMOSOME form and number in closely related animal groups are often remarkably constant. Indeed, for this reason, close similarity or great difference may be of interest taxonomically. In the sub-family Decticinæ (Orthoptera : Tettigoniidæ) four of the five Old World genera so far studied cytologically (*Decticus*, *Metrioptera*, *Gampsocleis* and *Platycleis*) are recorded as having a diploid number of 30+X rod-shaped chromosomes in the male (White, 1954). The American genera *Anabrus* (McClung, 1905, 1914), *Steiroxys* (Davis, 1908) and *Atlanticus* (White, 1941) differ considerably, however, having one or more pairs of autosomes metacentric. In the latter genus the X is also metacentric.

The single deviant in the Old World genera was *Pholidoptera*, where White (1941, 1954) claimed that an additional pair of autosomes was present in the species *Pholidoptera griseoaptera* (DeGeer), the new male number being $2n = 32+X$. Unfortunately, however, he gave no indication of the size range or centromere position of the new autosomal pair; neither did he publish any figures from which this could be deduced. Furthermore, he did not say how many meiotic cells of the two individuals he examined possessed clear counts of this number, no mitoses being observed.

A re-investigation of the cytology of this species was considered worthwhile, in view of the singular deviation of this record from those of the other related genera.

Five male imagines were collected near Welsh St Donat's, Glamorganshire, S. Wales, during the first week of August 1960. I am grateful to Mr T. Parsons for assistance on the collecting trip. The testes were vivisected in insect saline and, after removing the sparse fat body with needles, fixed in 1 : 3 acetic alcohol. Squash preparations were made, staining with acetic orcein.

## 2. OBSERVATIONS

### (I) *The standard karyotype*

In some 1000 cells at all stages of meiosis, taken from the five individuals, the normal male count was $n = 15\pm X (2n = 31)$, the number possessed by the four other related Old World genera.

All members of the complement are rod-shaped, representing either an acro- or telocentric organisation (*cf.* Marks, 1957). The 15 pairs of autosomes may be grouped into three size ranges, large (L1-2), medium (M3-8) and small (S9-15) (plate, fig. 1). The large and medium chromosomes are readily distinguished from one another, but the smaller members of the complement are very close morphologically and often cannot be identified with certainty. The X chromosome is unpaired in the male, and, as in all other Tettigoniidæ, is extremely large. When at a comparable stage of contraction it approaches the size of the L1.

### (ii) *Numerical deviants*

Seven cells were found in one individual exhibiting departures from the normal count, all apparently results of non-disjunction:

(*a*) Single cells at diplotene, diakinesis and first metaphase possessed two X chromosomes (*cf.* plate, fig. 5). Three early leptotene nuclei examined from the same individual also contained two X chromosomes (plate, fig. 9). All six cases must be a result of premeiotic non-disjunction. Indeed, though no mitoses were found in this individual, in one of the others about 50 mitotic anaphases were seen, in the majority of which, lagging separation of the X resulted in grossly elongated X bridges connecting daughter telophase groups. The large size of the X may at least play a part in this delayed separation. Such behaviour might be expected to lead, on occasion, to non-disjunction.

(*b*) In one other cell, two additional unidentifiable, small half-bivalents were apparently present at first anaphase, yielding a count of $2n = 32+X$ (text-fig. 1). Two possible explanations for this cell may be considered:

(1) The additional chromosomes represent a pair of B's, which had undergone normal bivalent formation and segregation (or been distributed to the two polar groups by chance). The complete absence of B's in all other cells of the five individuals makes this unlikely, however.

(2) The more likely alternative is that they arose by double mitotic autosomal non-disjunction, *i.e.* either non-disjunction of both homologues at the same premeiotic mitosis or non-disjunction of one or the other of a pair of homologues at two such successive mitoses, with subsequent bivalent formation and normal segregation (or else chance distribution).

Double mitotic non-disjunction has certainly been observed in the Acridid *Schistocerca gregaria* by John and Naylor (1961), where the largest pair of homologues has been found to be represented twice in one cell, with resulting quadrivalent formation.

Though non-disjunction may sometimes be a random process, there are two possible modes of non-random behaviour. On the one hand it may be *chromosome* specific, in which case one might expect the same chromosome to repeat the error, at least on occasion. Alternatively,

TEXT-FIG. 1.—First anaphase with one additional small half-bivalent at each pole. Sister chromatid bridge present, involving the M5. × 1750.

it may, by virtue of some aspect of structural or genetic organisation, be *homologue* specific. Under such conditions, comparable behaviour of both homologues would be more likely than not.

### (iii) *Bridges at first anaphase*

Recently, first anaphase bridges have been described in the grasshopper *Chorthippus brunneus* (John, Lewis and Henderson, 1960). They

TEXT-FIG. 2.—Four sister chromatid bridges in the same cell at first anaphase, involving (from left to right) the L2, M5, M6 and M4 bivalents. × 1750.

involve localised association of sister chromatids, most typically subterminally. Bridges of this type have also been observed here in *Pholidoptera*. As in *Chorthippus*, the largest members of the complement are most commonly involved, and the association is most characteristically subterminal (text-figs. 1, 2; plate, figs. 6, 7 10). Though it was not possible to determine the frequency from the small number of anaphases observed, up to four bridges have been seen in cells at this

stage (text-fig. 2). The bridges observed here possess two diametrically opposed characters not observed in *Chorthippus*:

1. The arm region adjacent to the centromere tends to be more readily uncoiled than the remainder of the arm, for this region was often found to be greatly elongated, the non-associated chromatid pulling away from its associated sister and taking its sister centromere with it. This tended to be asymmetrical, one side stretching considerably more than the other (plate, fig. 6). One bridge was found in which breakage of this elongating region had occurred, with complete loss of the centric end and the non-associated chromatid (plate, fig. 7).
2. Conversely, the non-bridging chromatids may on occasion remain tightly apposed to their bridging sisters, particularly at early anaphase (*cf.* plate, fig. 10).

The exact nature of these sister chromatid bridges has still not been elucidated, but they are in no way confined to *Chorthippus brunneus* and *Pholidoptera*. They have been observed in the following additional animal species:

| | | |
|---|---|---|
| (i) Orthoptera. | | |
| (*a*) Acrididæ | *Chorthippus albomarginatus* | Henderson (unpublished) |
| | *Chorthippus parallelus* | John and Henderson (unpublished) |
| | *Omocestus viridulis* | John and Lewis (unpublished) |
| | *Schistocerca gregaria* | John and Naylor (1961) |
| (*b*) Tettigoniidæ | | |
| | *Tettigonia viridissima* | Henderson (unpublished) |
| | *Conocephalus dorsalis* | Henderson (unpublished) |
| (ii) Diptera. | | |
| Tipulidæ | *Tipula brevispina* | Henderson (unpublished) |
| | *Tipula irrorata* | Henderson (unpublished) |

Furthermore, there are numerous figures in the literature from many different plant and animal groups which almost certainly represent this kind of bridge. Table 1 includes a few taken at random by a brief survey of some of the literature to indicate the range of organisms in which they may be present. A more exhaustive survey would doubtless reveal many more.

From this information it is readily apparent that close investigation may well reveal these bridges to be considerably widespread throughout both plant and animal kingdoms. The frequency with which they occur would be expected to vary from one organism to the next, possibly being higher in *Chorthippus* spp. than in the majority of animals and plants.

## 4. SUMMARY

1. The chromosome number in the male of *Pholidoptera griseoaptera* (DeGeer) (Orthoptera : Tettigoniidæ) is $2n = 30+X$, the number possessed by all other Old World genera of the sub-family Decticinæ.

TABLE I

| Author | Date | Organism | Figure | Reference |
|---|---|---|---|---|
| (i) *Plants* | | | | |
| Afify, A. | 1933 | *Aconitum stærkianum* | 26*a* | *J. Genet.*, *27*, 293-318 |
| Åkerman, Å. and Hagberg, A. | 1954 | *Avena sativa* | 36, 27, 38 | *Hereditas*, *40*, 438-452 |
| Darlington, C. D. | 1939 | *Fritillaria kamskatchensis* | 3*b*, *c*, *e* | *J. Genet.*, *37*, 341-364 |
| Dodds, K. S. | 1943 | *Musa* spp. | 9, 10, 11, 12 | *J. Genet.*, *45*, 113-138 |
| Doughty, L. R. | 1936 | *Agave amaniensis* | 4A | *J. Genet.*, *33*, 198-205 |
| Howard, H. W. | 1938 | *Raphanus sativus* × *Brassica oleracea* | 13, 33 | *J. Genet.*, *36*, 239-273 |
| Huskins, C. L. | 1927 | *Avena sativa* | 35 | *J. Genet.*, *18*, 315-364 |
| Larter, L. N. H. | 1932 | *Ranunculus acris* | 32 | *J. Genet.*, *26*, 255-283 |
| | | *Ranunculus repens* | 35 | |
| | | *Ranunculus ficaria* | 36, 37 | |
| Rees, H. | 1955 | *Secale cereale* | 2*a*, *b*, *c* | *Heredity*, *9*, 93-116 |
| Ribbands, C. R. | 1937 | *Lilium* × *testaceum* | 52 | *J. Genet.*, *35*, 1-24 |
| Richardson, M. M. | 1936 | *Lilium Martagon album* × *Lilium Hansonii* | 52 | *J. Genet.*, *32*, 411-450 |
| Upcott, M. | 1937 | *Tulipa gesneriana* (Inglescombe Yellow) | 28 | *J. Genet.*, *34*, 339-398 |
| | | *Tulipa Orphanidea* | 34*a* | |
| | | *Tulipa gesneriana spathulata* | 50 | |
| (ii) *Animals* | | | | |
| Ahmed, I. A. | 1941 | *Canis familiaris* | 18 | *Proc. Roy. Soc. Ed. B*, *61*, 107-118 |
| Belar, K. | 1929 | *Stenobothrus lineatus* | 9, 10, 68, 69, 70 | *Roux' Arch. Ent. Mech.*, *118*, 359-484 |
| Carothers, E. E. | 1913 | *Dissosteria carolina* | 59 | *J. Morphol.*, *24*, 487-512 |
| Crew, F. A. and Koller, P. C. | 1938 | *Sus scrofa* | 10*a*, *b* | *Proc. Roy. Soc. Ed. B*, *57*, 194-214 |
| Darlington, C. D. | 1932 | *Chorthippus elegans* | 13 | *Biol. Bull.*, *63*, 357-367 |
| Koller, P. C. | 1937 | *Homo sapiens* | 8*c*, *d* | *Proc. Roy. Soc. Ed. B*, *59*, 163-175 |

2. In seven cells from one individual, numerical deviants were found: (*a*) Six cells, three of which were at different meiotic stages, contained an extra X chromosome, a result of premeiotic non-disjunction. (*b*) One cell at first anaphase contained an additional small pair of half-bivalents, which probably resulted from non-disjunction of one of the small pairs of homologues.

3. First meiotic anaphase sister chromatid bridges of a type previously described in *Chorthippus brunneus* were found in many of the larger members of the complement. It is suggested that these bridges may well prove to be of widespread occurrence.

*Acknowledgments.*—I am indebted to Professor K. Mather and Dr B. John for helpful criticism in the preparation of this paper.

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*Plate*

FIG. 1.—Late diplotene : 15AA+X. ×1250.

FIG. 2.—First metaphase : 15AA+X. ×1250.

FIG. 3.—Second metaphase, X absent : 15A. ×1250.

FIG. 4.—Diakinesis : 15AA+X. ×1250.

FIG. 5.—First metaphase : 15AA+XX. ×1250.

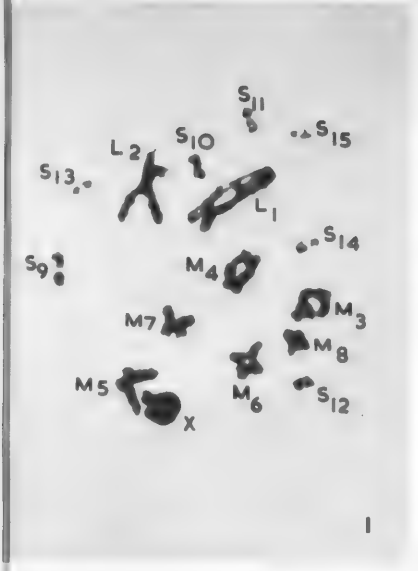
FIG. 6.—Asymmetrical elongation of the associated arms in a sister chromatid bridge at late first anaphase. ×1250.

FIG. 7.—First anaphase bridge in which upper sister chromatid and centromere of associated chromatid have been detached by breakage. ×2000.

FIG. 8.—Spiral structure of X chromosome from early diplotene nucleus. ×2000.

FIG. 9.—Early leptotene nucleus with two heteropycnotic X chromosomes. ×1250.

FIG. 10.—First anaphase with sister chromatid bridges involving the L1, L2 and M6 (one in M3 having recently broken). Close lateral association of the non-associated sister chromatids, with rotation, is exhibited by the L2. ×1250.

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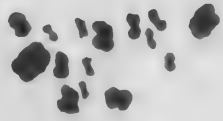

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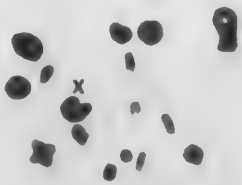


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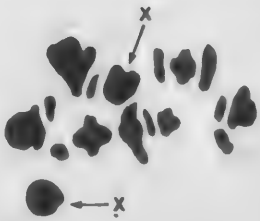


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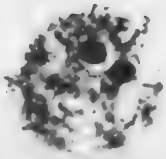

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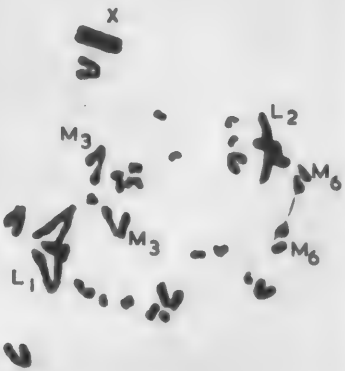


10

# ANOMALOUS CHROMOSOME BEHAVIOUR IN THE GERM LINE OF *SCHISTOCERCA GREGARIA*

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Received 1.iii.61

## 1. INTRODUCTION

One of the standard methods of analysing biological normality is by the study of anomaly. This method is not without limitations; in particular its successful application depends upon correctly relating the abnormal to the normal. But it can often provide crucial information which is unobtainable in any other way. For example a whole series of chromosome anomalies, recently summarised and extended by Brown and Zohary (1955), collectively provide critical evidence for the chiasmatype hypothesis.

The study of chromosome anomalies is especially instructive because such anomalies may sometimes be the starting point for evolutionary change. Not only does their study lead to a better understanding of the mechanical properties of the chromosomes but it often reveals the potentialities of a chromosome complement for change.

## 2. MATERIAL AND TECHNIQUE

All observations were made on 24 male sibs of the desert locust *Schistocerca gregaria* (Forskål). These were derived from a mating between two isolated members of a stock culture maintained by the Anti-Locust Research Centre. This culture was started in 1946 from egg pods obtained in British Somaliland. In 1953 and 1957 two additional batches of wild adults were added to the stock and throughout the life of the culture the locusts have been kept in conditions of maximum crowding. There are approximately four generations per annum and at each generation there is a 60-80 per cent. mortality prior to mating. Numbers are even further reduced in supplying specimens for research purposes and the culture is then maintained from the survivors.

Following vivisection in insect saline the testis follicles were freed from the investing fat body, fixed in 1 : 3 acetic alcohol and subsequently squashed in acetic orcein.

## 3. THE NORMAL COMPLEMENT

Although several workers have previously used males of this species for cytological purposes little attention has been paid to the overall properties of the standard complement. For this reason it seems worth summarising these before dealing with the anomalies:

(i) The chromosome complement of the male in this species is $2n = 22+X$ (plate 1, figs. 1-6). The autosomes can be divided into three groups on the basis of their relative sizes; these

include three pairs of large, five pairs of medium and three pairs of small homologues (plate 1, figs. 3-5). The X chromosome is probably the second largest in size (table 1).

(ii) All twenty-three chromosomes are acrocentric (plate I, figs. 2-4).

(iii) The spindle tends to be hollow in all cells of the germ line. This tendency is not, however, absolute for at mitosis and especially at meiosis the smaller autosomes and the univalent sex chromosome frequently lie in the centre of the spindle (plate I, figs. 3, 4, 6 and 13). This agrees with their relative size and valency relationships respectively (*cf.* Lewis and Scudder, 1958).

TABLE 1

*Mean linear dimensions of the basic complement with their standard deviations*

| Stage | Mean length in micra | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | L1 | L2 | L3 | M4 | M5 | M6 | M7 | M8 | S9 | S10 | S11 | X |
| Mitotic metaphase (mean from 16 nuclei) | 7·4 ± 0·1 | 6·7 ± 0·2 | 5·8 ± 0·1 | 4·9 ± 0·1 | 4·3 ± 0·1 | 3·9 ± 0·1 | 3·6 ± 0·1 | 3·3 ± 0·1 | 2·4 ± 0·1 | 2·1 ± 0·1 | 1·9 ± 0·1 | 6· ± 0· |
| Pachytene (mean from 25 nuclei) | 71·5 ± 2·6 | 58·8 ± 2·7 | 47·7 ± 2·1 | 39·9 ± 1·8 | 30·8 ± 1·2 | 26·6 ± 1·1 | 24·6 ± 1·0 | 21·8 ± 1·0 | 15·7 ± 1·0 | 14·2 ± 1·0 | 12·4 ± 1·0 | ·4 ± 0· |

(iv) The unpaired X chromosome is allocyclic with respect to the autosomes. It is negatively heteropycnotic at mitosis (plate I, figs. 1 and 2) and at first metaphase of meiosis (plate I, fig. 6) and positively heteropycnotic at pre-meiotic interphase, at first (plate I, fig. 5) and second prophase of meiosis and in the first post-meiotic interphase. At metaphase-I the X is rather variable in appearance. Usually it is completely negatively heteropycnotic. But in other cases either the centric end alone (compare *Chorthippus*; John, Lewis and Henderson, 1960) or infrequently both ends (compare (*Pyrgomorpha*; Lewis and John, 1959) of the X retain their prophasic appearance.

(v) The autosomes, on the other hand, show a differential behaviour within themselves during pachytene when their centric end segments condense in advance of the main chromosome thread (plate I, fig. 5; plate II, fig. 10). This, like the tendency for the development of a hollow spindle, is a property which is widely distributed in orthopteran species.

## 4. THE ANOMALIES

Five types of meiotic anomaly were observed. These were as follows :

(i) Chromatid bridge formation at AI.
(ii) The occurrence of achromatic gaps at pachytene-diplotene.
(iii) Univalent formation.
(iv) Multiple formation by interchange.
(v) Multivalent formation following mitotic non-disjunction.

Anomalies (i) and (ii) were found in the same individual (No. 3) and (i) was also found in individual No. 1. The remainder occurred in distinct individuals.

The first two of these need little discussion. The chromatid bridges were of the type we have recently discovered in *Chorthippus brunneus* (John, Lewis and Henderson, 1960) involving an interstitial association between sister chromatids in one of the long chromosomes (plate I, fig. 7). Such bridges were found in single anaphase cells of two individuals, Nos. 1 and 3 respectively; achromatic gap formation also occurred in No. 3. Here 48 per cent. of the pachytene cells examined included chromosomes with marked gaps (plate II, figs. 8 and 9). Seventy per cent. of the affected cells had two such chromosomes per nucleus and the remainder one. These gaps occurred in either the long or medium chromosomes only and involved both chromatids of either one or both homologues of a bivalent. In the latter case corresponding loci were always concerned. Diplotene cells with similar gaps were also found in this individual, but were not evident at the later meiotic stages; neither did they lead to any apparent subsequent abnormality. Comparable achromatic gaps were seen in other individuals of this sibship but here their frequency never exceeded 1 per cent. We have also seen such exceptional behaviour in *Chorthippus brunneus*.

Neither of these two anomalies gives us any useful information about the inherent properties of the complement because, as yet, wé do not understand their nature. But the individual with univalents tells us something about the control of bivalent formation and the two remaining anomalies give us some insight into the principles which govern karyotyptic change.

## 5. THE CONTROL OF BIVALENT FORMATION

Univalents were recorded in five of the individuals examined. In four of these their frequency did not exceed 2 per cent. but in individual (N1) 14 per cent. of the 415 meiotic cells scored contained either one or, less usually, two pairs of univalents. These occurred predominantly in the S9-S11 classes, less frequently in the M8 chromosomes (plate II, figs. 10-12). The occurrence of univalents in this individual raises two points of particular interest. First, to what extent are the chiasma

frequencies of the three size classes of chromosomes affected in the cells with univalents? Secondly, does the pattern of chiasma frequency differ in the normal nuclei of this individual as compared with normal cells in unaffected sibs?

TABLE 2

*Comparison of chiasma frequency distributions in 55 cells with and without univalents in individual N1*

| Chromosome size group | Mean cell chiasma frequency | | $t$ | P |
|---|---|---|---|---|
| | Cells with univalents | Normal cells | | |
| Long (L1-L3) . . . | 6·47±0·15 | 6·86±0·12 | 1·99 | 0·05-0·02 |
| Medium (M4-M8) . . | 6·19±0·17 | 7·53±0·15 | 2·71 | 0·01-0·001 |
| Short (S9-S11) . . | 2·15±0·06 | 3·00±0·00 | 15·68 | ≪0·001 |

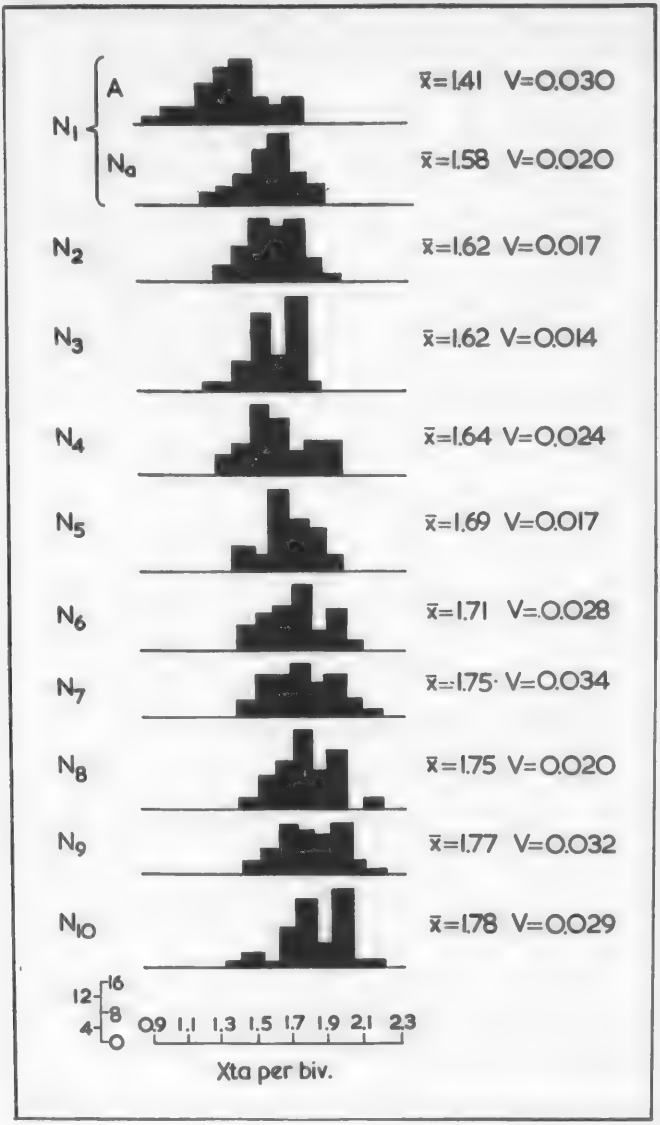
To resolve the first of these questions a total of 110 meiotic nuclei were scored in the anomalous individual; 55 of these contained univalents and 55 were normal. In each nucleus the chiasma frequency of the three size classes of chromosomes were treated separately and the data is summarised in table 2. A $t$ test for the differences in means between cells with and without univalents is given in column three of this table and in all cases the differences are significant.

TABLE 3

*Analysis of variance of the difference between mean chiasma frequencies in normal cells of the anomalous individual (Na) and normal cells of nine sibs (N2-10). Fifty-five cells were scored in each of the ten individuals.*

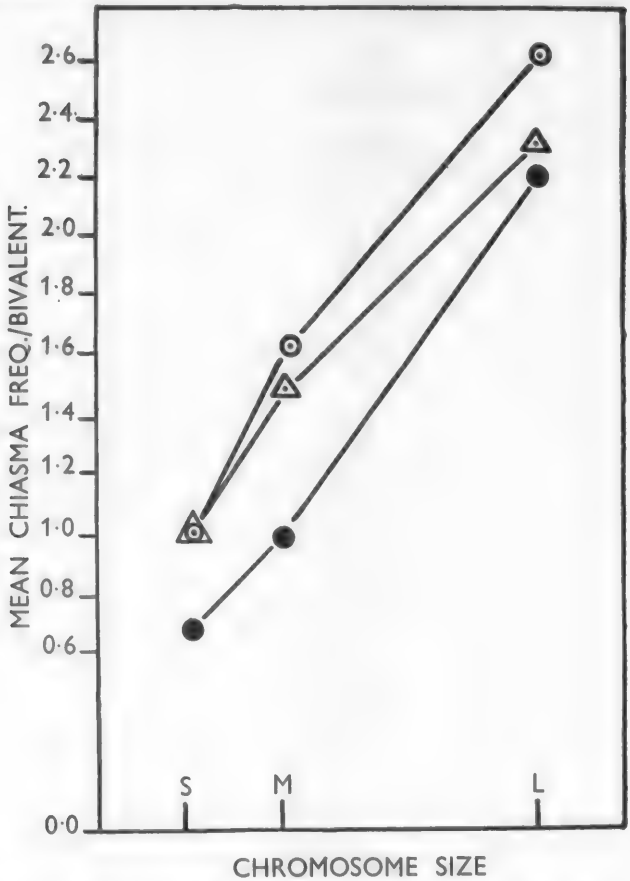
| Item | N | M.S. | P |
|---|---|---|---|
| 1. Na V N2-10 . . . | 1 | 90·69 | 0·20-0·10 |
| 2. Between N2-10 . . . | 8 | 27·88 | <0·001 |
| 3. Error (between cells) . . | 540 | 2·85 | ... |

The second question was resolved by comparing the difference between the mean chiasma frequency in 55 normal cells of the anomalous individual (N1) with that of 55 cells from each of nine normal sibs of the same age group (N2-N10) giving a total of 550 observations and thus 549 degrees of freedom. Of these one goes to the comparison between N1 and N2-10, 8 are concerned with the variation between the 9 normal sibs leaving 540 for the error item (table 3). This comparison shows that the 9 normal sibs differ amongst themselves as far as chiasma frequency is concerned (text-fig. 1). Because




TEXT-FIG. 1.—Distribution of chiasma frequencies in 55 cells with univalents (A) and 55 normal (Na) cells of the asynaptic individual (N1) and in 55 cells from each of nine normal sibs (N2-N10).

of this the appropriate test of significance for the differences between the normal nuclei of the anomalous individual and that of the pooled 9 normal sibs depends upon a comparison between the first and second item mean squares in table 3. Such a comparison demonstrates that




TEXT-FIG. 2.—Mean chiasma frequency per bivalent type in each of the three size classes of chromosomes taken from nine normal individuals, N2-N10, (⊙) and from asynaptic cells, A, (●) and normal cells, Na, (△) of an asynaptic individual of the same age and sibship. The chromosome size relationships have been calculated from mitotic chromosome lengths.

the mean chiasma frequency of these two classes of normal nuclei does not differ significantly (text-fig. 2).

Now univalent formation in a diploid species may result from a variety of causes, both genotypic and environmental, and the precise cause is not always easily determined. In a first analysis, however,

one must attempt to distinguish between true failure of pairing (asynapsis, *e.g.* Ross *et al.*, 1960) and chiasma failure following successful pairing (desynapsis). This distinction is one that has not always been, or could be, correctly made. Indeed in so-called " asynaptic " maize pachytene pairing looks complete (Beadle, 1933) so that here univalent formation is a consequence of desynapsis. The same appears to be true in many " asynaptic " plants (*cf.* Li, Pao and Li, 1945).

Such behaviour forces us to admit that while pairing is a necessary pre-requisite for exchange it is not in itself a sufficient cause for crossing over and that there is a need to distinguish between effective and non-effective pairing. Thus Darlington and Haque (1955) described an abnormal plant of *Allium ascolonicum* in which, while chromosome pairing was complete, a large proportion of the p.m.c.'s were desynaptic. The marked decrease in the number of chiasmata in these cells was explained by a reduction of coiling to about one-half that normally found. This then is a case in point; it would appear that it is a failure in coiling, not pairing, which is responsible for the decreased chiasma formation and hence desynapsis. Clearly there are factors which can impede the formation of chiasmata without exerting any visible effect on pairing.

In the case under consideration univalents were present at pachytene (plate II, fig. 10). This evidence is not as compelling as it might be because the small chromosomes are frequently in advance of the remainder of the complement with respect to chromosome repulsion and the opening out of the pachytene bivalent (plate I, fig. 5; plate II, fig. 10). However, further support for a possible asynaptic mode of origin of the univalents in this case comes from some observations recently made in our laboratory on the effect of high temperature treatment on chiasma formation in *S. gregaria*. These observations are as yet incomplete but Mr S. A. Henderson, who is carrying out the work, allows us to make the following significant points:

(i) In the heat treated specimens varying numbers of univalents are present but the small chromosomes are generally first to be affected. Univalent formation in this case can definitely be correlated with a failure of pairing and this applies to all classes of chromosomes. In extreme cases there may be total asynapsis.

(ii) Where partial asynapsis occurs the chiasma frequency of the longest chromosomes is significantly reduced and is accompanied by an increase in the differential distance. In extreme cases the chiasmata are distally localised.

Asynapsis is also supported by the fact that the univalents in our case show no tendency to secondary association (*cf.* Thermann, 1951; Riley and Chapman, 1957).

On the other hand we have the evidence that there is a general reduction in chiasma frequency in the univalent containing cells. As a

minimum hypothesis it would appear more likely that both univalent production and chiasma frequency reduction are governed by the same cause—a failure of effective pairing. From a cytological point of view, however, those homologues which succeed in forming bivalents in the univalent containing cells appear to be completely paired; chromosome contraction also looks normal (plate II, fig. 10). Obviously, however, although pachytene behaviour looks normal it must be abnormal in some way since there is a significant decrease in chiasma frequency for all chromosome classes irrespective of whether or no these classes are represented as univalents (table 2). It is of course possible that partial pairing by attraction is extended by torsion forces (see McClintock, 1933) with only the former leading to effective pairing.

Our provisional conclusion then is that we are dealing here with a case of asynapsis due to an absence of pairing or a reduction in its efficiency. The former is expressed as univalent production in the smallest chromosomes and the latter in a reduced chiasma frequency in those which succeed in forming bivalents. But these processes have involved only 14 per cent. of the cells analysed. The remaining cells are all normal in pairing behaviour and do not differ significantly in their chiasma frequency from that of normal sibs. It is interesting to note, however, that the mean chiasma frequency of these normal cells is halfway between that of the univalent containing cells and the average mean of 9 normal sibs (text-fig. 1). It would appear, therefore, that the cells with univalents are merely an extreme expression of a continuous distribution about a lower mean, but since one chiasma is the minimum compatible with bivalent production a pronounced visible distinction necessarily arises between those cells which do and those which do not contain univalents. This distinction can be treated as a meristic difference because the presence of univalents is not consistent with regular segregation.

This situation provides an instructive comparison to that described by Rees (1957) in *Locusta migratoria*. Here the same number of chromosomes are present as in *Schistocerca* and a similar range of chromosome size occurs within the complement. In one individual Rees found extensive univalent formation with the highest proportion of univalents in the long and long medium chromosomes. Clearly the causes of reduced chiasma frequency are different in these two cases. In the one, *Schistocerca*, the reduction is general affecting all the chromosomes, though not equally so, while in the other, *Locusta*, it is differential affecting the long chromosomes preferentially.

Although he did not analyse pachytene cells of *Locusta* in detail, Rees (personal communication) is of the opinion that in his case unpaired chromosomes, or chromosome regions were not present to any significant extent at this stage. It is possible then to reconcile these two divergent instances on the assumption that Rees was dealing with a case of desynapsis. In *Neuroptera* Suomalainen (1952) has found that

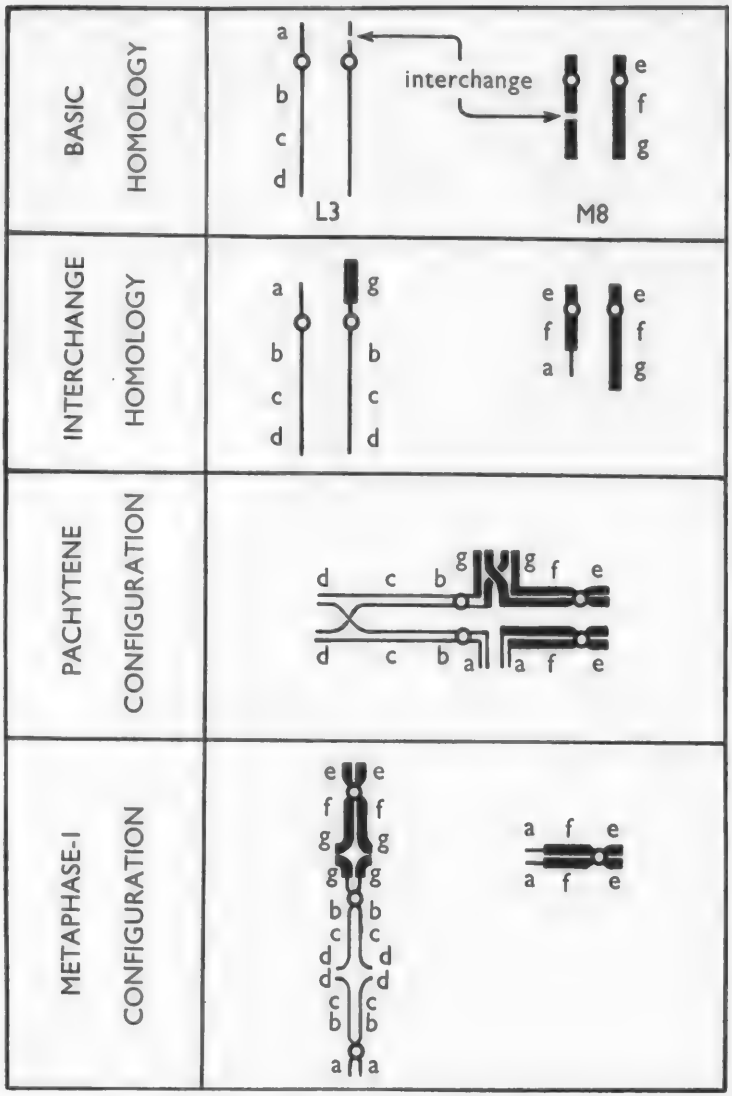
pachytene pairing is complete but that nevertheless chiasmata are localised distally. Significantly, however, polarisation of the leptotene-zygotene nuclei determines that pairing begins at the chromosome ends. Here it would appear that chromosome segments which pair first are preferred in chiasma formation presumably because they reach the appropriate stage for exchange before the later paired regions. In at least some instances of asynapsis or desynapsis there are definite indications that general disturbances of timing relations are involved (Rees, 1952; Darlington and Haque, 1955). Certainly alterations in the time available for pairing appears to be one of the prime variables of meiosis (Darlington, 1940). We have already commented on the fact that in *Schistocerca* the small chromosomes are frequently in advance of the remainder in their prophase behaviour. Perhaps in complements with such marked size differences chromosomes which, by virtue of their small size, pair first are also preferred in chiasma formation. Under these circumstances if the chiasma frequency falls below its normal range the small chromosomes will be at an immediate advantage in forming chiasmata.

However these contrasting observations are ultimately resolved they serve to emphasise the complexity of the processes involved in the control and maintenance of bivalent development.

## 6. THE BASIS OF KARYOTYPE CHANGE

In individual No. 2 a cell was found containing nine bivalents, one trivalent and one univalent in addition to the unpaired X (plate II, fig. 13). Such an association would follow a reciprocal translocation between the L3 and M8 chromosomes in which the interchange has involved the terminal segment of the short arm of the L3 and the distal portion of the long arm of M8 (text-fig. 3). A single cell containing ten bivalents, one quadrivalent and the sex univalent occurred in individual No. 9 (plate II, fig. 14). Quadrivalent formation in this instance must have been the consequence of simultaneous non-disjunction in both homologues of one of the L chromosomes at the last pre-meiotic mitosis.

Reorganisation of the chromosome complement during evolution depends upon two events: first, the occurrence of both structural and numerical anomalies and secondly, the subsequent survival of these. To a large extent it has been assumed, explicitly or implicitly, that all types of chromosome mutation probably occur in all organisms at low frequencies but that few survive to be selectively incorporated into the genetic system. While it is debatable to what extent this statement is generally applicable there can be little doubt that, as far as orthopterans are concerned, the evidence, with one qualification, favours acceptance of this idea. This qualification depends upon the fact that while the chromosome system may have attained stability under particular breeding conditions it may become unstable if the breeding system departs from its characteristic structure (*cf.* Rees,




TEXT-FIG. 3.—Derivation of the L3-M8 interchange association of three (*cf.* plate II, fig. 13).

1955; Lewis and John, 1959). Indeed we suggest that the situation observed in this single sibship is not fortuitous and that these several anomalies all result from a breakdown of normal genotypic control and must be regarded as instances of correlated instability. This instability in turn is a reflection of the unusual breeding structure of the culture from which the sibship was derived.

With this qualification in mind there is no doubt that most of the major classes of structural and numerical changes have been, or can be, demonstrated to occur spontaneously in the germ line of orthopterans (White, 1959; Lewis and John, unpublished). In this present account we can add isolated examples of aneuploidy and interchange. There is, however, equally no doubt that few of these, including our two instances, can be expected to survive. There are of course good reasons why this should be so. Chromosome behaviour is normally self-regulating in the sense that anomalies, because they lead to mechanical disturbance at meiosis, necessarily produce a reduction in fertility.

Structural and numerical hybrids may, therefore, produce segregational sterility because of the unbalance of their meiotic products. But the extent to which they do so is largely a reflection of their own genetic organisation, depending in particular on the cyto-morphological characteristics of their chromosomes and more especially upon their properties of chiasma formation. Any mechanism which determines the formation of balanced gametes or which, in animals, leads to the incapacity of unbalanced ones, will favour the survival of the new chromosome type. How this operates is particularly well illustrated by the Diptera which thus far appear to be unique among animals in the frequency with which paracentric inversions occur in wild populations. Here there are several mechanical peculiarities of the meiotic system which facilitate a toleration for this particular category of change (Brito da Cunha, 1960).

The inherent organisation of the karyotype thus provides for its own change by facilitating or limiting both the types of chromosome reorganisation which can occur and those which can be expected to establish themselves. The case against the establishment of aneuploid types is well known and recently we have considered in detail the special problems which limit the survival of interchange multiples in complements which, like *Schistocerca*, are completely acrocentric in organisation (John and Lewis, 1960).

## 7. SUMMARY

1. A series of five types of chromosome anomalies have been found in the meiotic cells of a single sibship of the desert locust *Schistocerca gregaria*.

2. Three of these involved only single cells in distinct individuals including one instance of both interchange and pre-meiotic non-disjunction and two cases of chromatid-bridge formation at AI.

3. The remaining two anomalies were more extensive, though both were confined to single individuals. One of these took the form of peculiar achromatic gaps at prophase I in conjunction with Anaphase-I chromatid bridge formation. The other involved chromosome asynapsis.

4. We are inclined to interpret these several anomalies as correlated changes in nuclear phenotype dependent upon the production of a novel genotype within the sibship. This is perhaps determined by the inbred nature of the culture from which the sibship was derived.

5. The anomalies themselves have no apparent future in heredity or evolution though of course genetically identical cells of normal phenotype may survive to perpetuate their genetic basis.

*Acknowledgments.*—We are grateful to Professor K. Mather for critical reading of the manuscript. We also wish to thank Mr Phillip Hunter-Jones of the Anti-Locust Research Centre for supplying the material.

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*Plate I*

All acetic orcein squash preparations.

FIG. 1.—Mitotic prophase ; X to right of autosomes and undercondensed. × 1500.

FIG. 2.—Mitotic metaphase ; negatively heteropycnotic X at seven o'clock. × 1500.

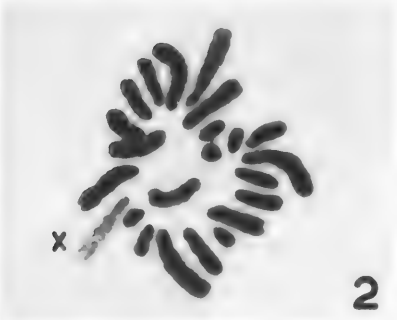
FIGS. 3 and 4.—Metaphase II in cells with (fig. 4) and without (fig. 3) the X to show size range of the complement. × 1500.

FIG. 5.—Pachytene ; note pre-condensation of autosomal centric ends and precocity of separation of the 3 small pairs of homologues. Positively heteropycnotic X at nine o'clock. × 900.

FIG. 6.—Metaphase I ; note arrangement of negatively heteropycnotic X and the three S bivalents inside the hollow spindle. × 900.

FIG. 7.—Anaphase I ; note lagging L2 bivalent with a chromatid-bridge. × 1500.

X
1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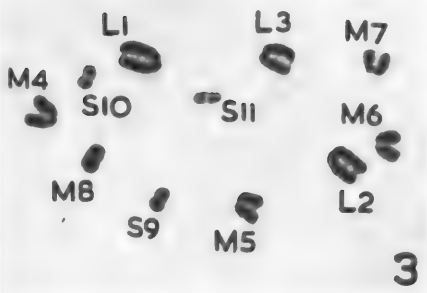
L1
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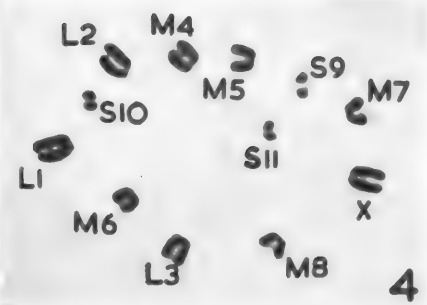
L2
S9
M5
3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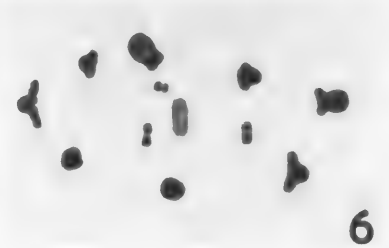
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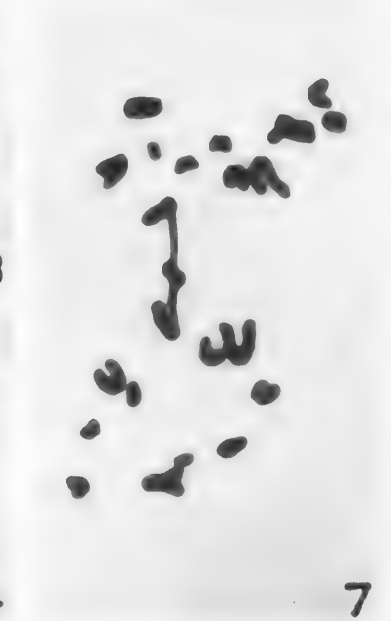
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*Plate II*

All acetic orcein squash preparations.

FIG. 8.—Early diplotene ; note pronounced heteromorphic gap in the L3 bivalent. ×900.

FIG. 9.—Enlargement of the anomalous chromosome of fig. 8. ×2750.

FIG. 10.—Pachytene ; note two S10 univalents at seven o'clock. ×900.

FIG. 11.—Diakinesis ; note two S9 and two M8 univalents. ×900.

FIG. 12.—Diakinesis ; note two S11 univalents. ×900.

FIG. 13.—Metaphase I ; this nucleus contains an L3-M8 terminal interchange (CIII+I+9II+X). The interchange trivalent is in indifferent orientation.

FIG. 14.—Diakinesis ; here the nucleus contains a ring-of-four multivalent (RIV+10II+X) which is the consequence of pre-meiotic non-disjunction. ×900.

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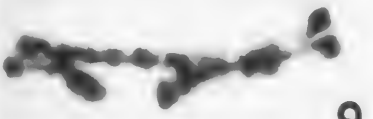
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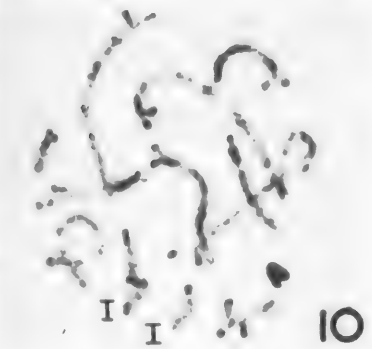
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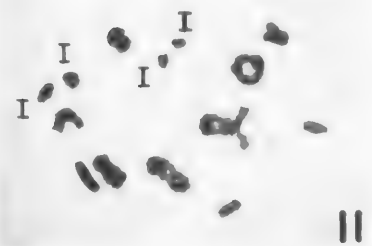
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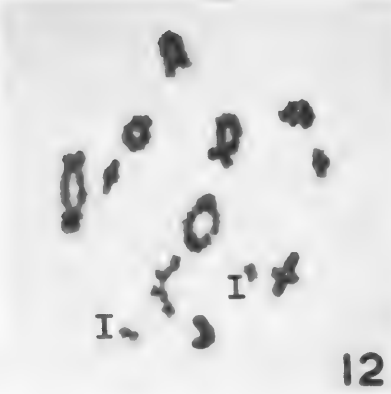
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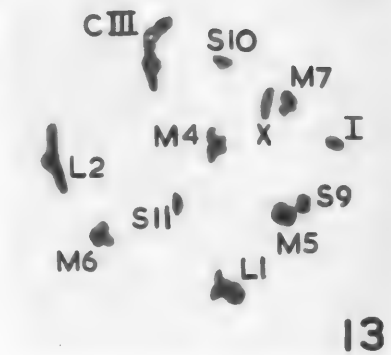
CIII
SIO
M7
M4
X
I
L2
S9
SII
M5
M6
LI
13

RIV
14

# EFFECTS OF DISRUPTIVE SELECTION

## IV. GENE-FLOW AND DIVERGENCE

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Received 8.iii.61

## 1. INTRODUCTION

In his general discussion of disruptive selection, Mather (1955*a*) argued that it is to be expected to have two different kinds of consequence. In so far as different interdependent classes of phenotype are selected, it is to be expected that disruptive selection, given appropriate genetic variation, will produce a polymorphic population. It has been shown experimentally (Thoday, 1958*a*, 1959, 1960; Thoday and Boam, 1959) that this is so. On the other hand, if different independent phenotypes are selected, disruptive selection might result in the development of physiological isolation between them.

Between these two extremes there are intermediate situations in which a population is to some extent split into two parts selected for different characteristics, but in which the split is not permitted to be complete. Such situations should be very revealing, for they should permit experimental assessment of the relative effectiveness of disruptive selection pressure in promoting and of gene-flow in preventing genetic divergence of parts of a population from one another. The need for some such experimental assessment of the importance of isolation hardly needs stressing, as Mather (1955*b*) pointed out in another paper.

Thoday and Boam (1959) have already shown that isolation is not a prerequisite of divergence under divergent selection pressures. Their experiment, however, involved an extreme amount of gene-flow for, in each generation, 50 per cent. of the genes in each half of their population were derived from the other half. This is twice the gene-flow as would be involved in random mating. The present experiments were started with a view to assessing the amount of divergence possible with 25 per cent. gene-flow, the formal equivalent of random mating. A preliminary account of some of the results has already been published (Millicent and Thoday, 1960).

## 2. MATERIAL AND METHODS

Selection was for sternopleural chaeta number, and the lines were initiated from the same wild stock (Dronfield) of *Drosophila melanogaster* as that used by Thoday (1958*a*, 1959) and Thoday and Boam (1959) in the earlier experiments on disruptive selection. Methods of maintenance, the number of flies assayed (20 of each sex of

* Work carried out while holding a D.S.I.R. studentship and accepted for the degree of Ph.D. of Sheffield University.

each culture) and the proportion of flies selected (1 in 20) were the same. There were 4 single pair cultures per line per generation.

Three mating systems were used (table 1). The first system gives 50 per cent. gene-flow between the high and low halves of the population and is identical with that of Thoday and Boam (1959) except that males instead of females were used as the migrators through whom the gene-flow from high to low halves of the population occurred. The second mating system is a modification of the first designed to reduce gene-flow to 25 per cent. The third mating system gives 0 per cent. gene-flow between the high and low halves. The lines maintained with this latter system will for convenience be referred to as lines or populations, though strictly, of course, each is composed of two separate populations, one under directional selection for high and the other under directional selection for low chaeta number.

TABLE 1

*The mating and selection schemes giving 50 per cent. and 25 per cent. and 0 per cent. gene exchange*

| Population | Culture | ♀ Parent | ♂ parent in generation | | | |
|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 etc. |
| 50 per cent. gene-exchange | A | AH | CH | DH | CH | DH |
| | B | BH | DH | CH | DH | CH |
| | C | CL | AL | BL | AL | BL |
| | D | DL | BL | AL | BL | AL |
| 25 per cent. gene-exchange | A | AH | BH | DH | BH | DH |
| | B | BH | CH | AH | CH | AH |
| | C | CL | DL | BL | DL | BL |
| | D | DL | AL | CL | AL | CL |
| 0 per cent. gene-exchange | A | AH | BH | BH | BH | BH |
| | B | BH | AH | AH | AH | AH |
| | C | CL | DL | DL | DL | DL |
| | D | DL | CL | CL | CL | CL |

H indicates the highest, and L the lowest flies chosen from a sample of twenty. The letters A-D indicate the female sub-line from which the designated flies are taken. Only males are chosen to migrate.

The first and third mating systems were used primarily as controls with which the 25 per cent. gene-flow lines could be compared.

Two replicate lines were maintained with each mating system. The lines will be designated 50/A, 50/B, 25/A, 25/B, 0/A, and 0/B, and were initiated as follows. Twelve four-pair cultures of the Dronfield wild stock were set up. These we will refer to as X a-d, Y a-d and Z a-d, as they were used in three groups of four cultures each. The four single pairs of flies used to establish Line 50/A were taken from the four cultures Xa, Xb, Xc, Xd ; so were those used to establish Line 25/B. Lines 25/A and 0/B were established from the Y cultures. Lines 0/A and 50/B were established from the Z cultures. Thereafter Lines 50/A, 25/A and 0/A were cultured coincidentally. Lines 50/B, 25/B and 0/B were likewise cultured coincidentally, but in weeks alternating with those in which the A lines were cultured.

These details are given because they are relevant to the comparison of the lines' behaviour. The basic X, Y and Z groups of culture had different within sex and culture variances, 2·375 for X, 2·085 for Y and 1·735 for Z, the first and last figures being significantly different ($p<0.05$).

It will be noted that, in terms of origin, Lines 25/A and 0/B are a comparable pair, whereas in terms of culture times 25/A is to be compared with 0/A.

## 3. RESULTS

### (i) *Divergence*

The results are presented in two forms. Figs. 1-4 illustrate the mean chaeta numbers of the 40 flies assayed in each of the four cultures of

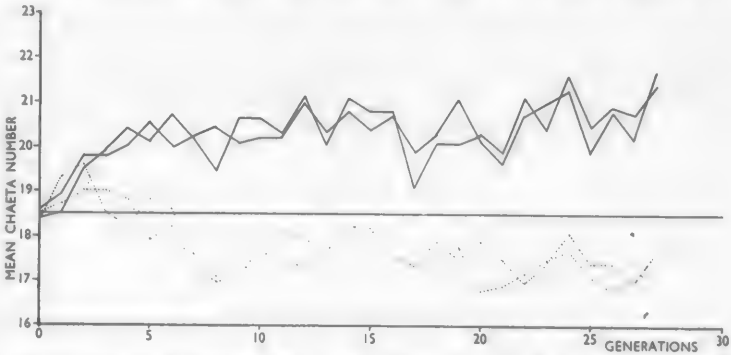


FIG. 1.—Mean chaeta numbers for the 4 female sub-lines of o/A. Solid lines, high cultures and dotted lines low cultures in this and figs. 2-4.

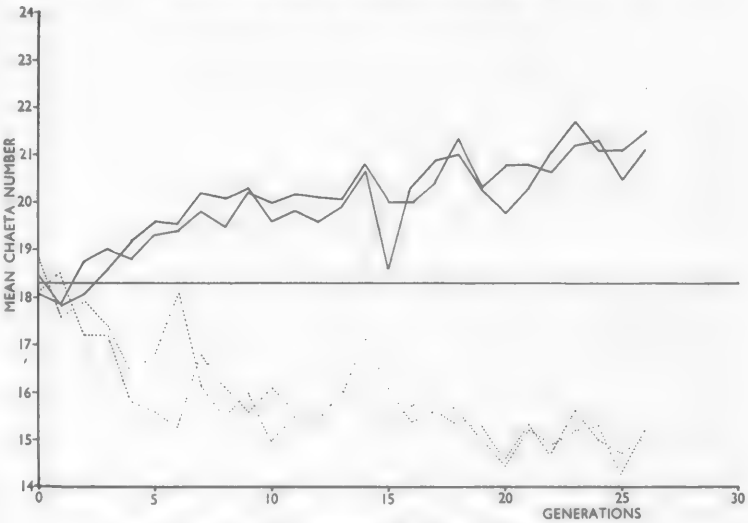


FIG. 2.—Mean chaeta numbers for the 4 female sub-lines of o/B.

each of the 0 per cent. and 25 per cent. gene-flow populations, and fig. 5 illustrates the rates of divergence in the six " lines " and permits comparisons to be made more readily.

The high and low cultures diverged in mean in each of the six lines. Divergence of the 0 lines was more rapid than in the other

lines. The effects of gene-flow in the 25 per cent. populations can be seen in the zigzag behaviour of the pairs of cultures, especially after a considerable degree of divergence had occurred.

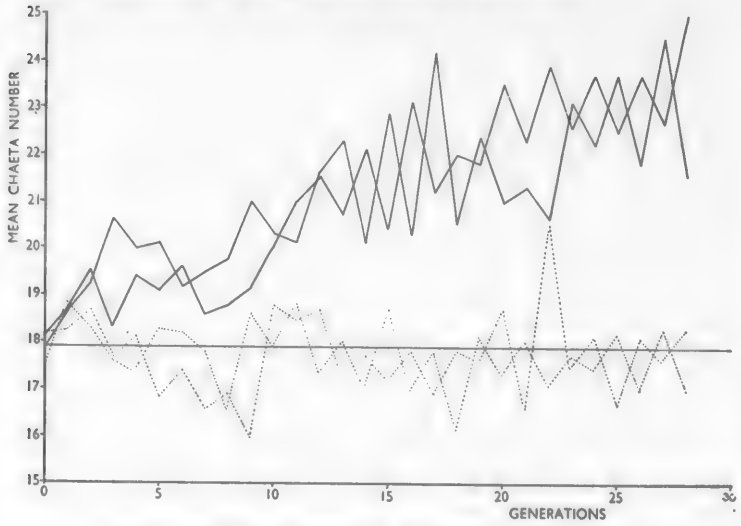


FIG. 3.—Mean chaeta numbers for the 4 female sub-lines of 25/A.

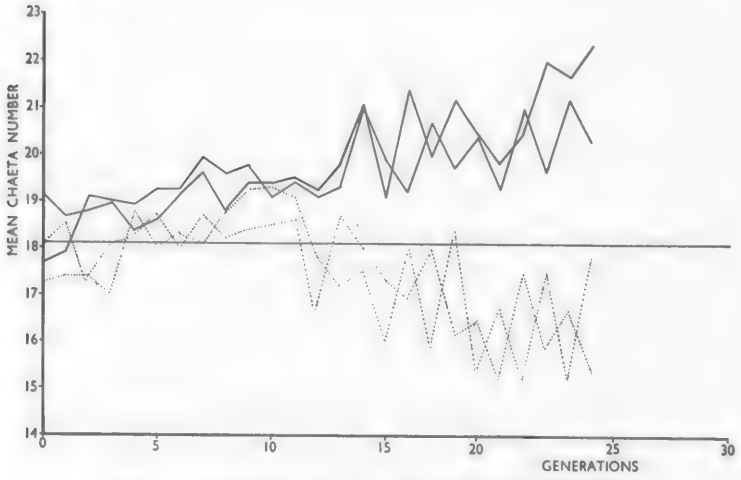


FIG. 4.—Mean chaeta numbers for the 4 female sub-lines of 25/B.

Divergence was greatly impeded by 50 per cent. gene-flow, though some positive difference was maintained. This was not as large as in the population described by Thoday and Boam (1949), neither is it

as consistent. That the 50 per cent. gene-flow populations were less successful than that of Thoday and Boam may be related to the use of males instead of females as migrants in the present population, which would permit any extreme recombinant chromosomes selected on either side to break down more readily by further recombination. It may on the other hand be related to the nature of the genetic variance available in the foundation cultures. The Dronfield wild stock may well have lost some genetic variance in the 2½ years between the initiation of Thoday and Boam's experiment and these, or accidental sampling may be responsible.

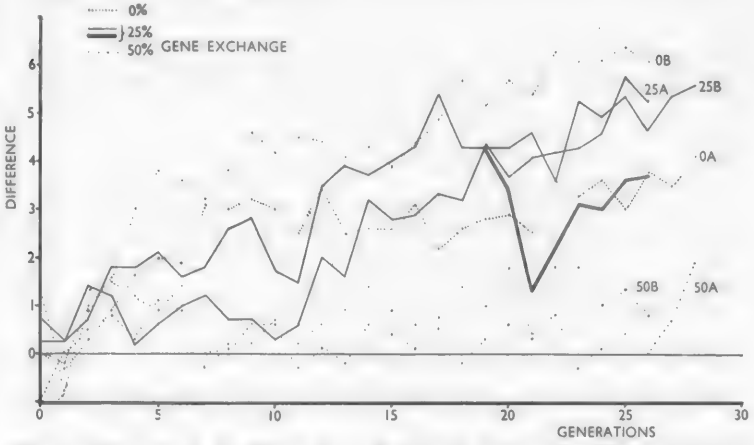


FIG. 5.—Divergence in the different lines. Each graph represents the difference between the mean chaeta numbers of the high and the mean chaeta numbers of the low cultures of the line. The heavy line is the curve for the negative assortative mating sub-line of 25/B (see p. 215).

The 0 populations diverged reasonably readily, but a plateau seems to have been reached rather soon, no doubt partly because of the small population size. Each half of each population is of course maintained by only two pairs of flies per generation and this involves fairly heavy inbreeding.

It is the 25 per cent. gene-flow populations that provide the main interest. They diverged more slowly than the 0, which is hardly surprising. But in time they diverged as much as the 0 populations which is. The comparison that seems most legitimate is that between 0/A and 25/B, which originated from the same cultures. The ultimate divergence of 25/B was greater than that of 0/A. 0/B and 25/A showed similar ultimate divergence, and, though they originated from different cultures they add to the evidence. The two 25 per cent. gene-flow populations taken together diverged a little more than the two 0 per cent. populations. It does not seem that the 25 per cent. gene-flow of the type arranged in these experiments seriously limits the amount of divergence that can occur.

### (ii) *Variance and heritability in the selected lines*

Fig. 6 illustrates the within sex and culture mean squares of the different lines. There is no suggestion of change with generation in the 0 per cent. gene-flow lines, little evidence of change in the 50 per cent. gene-flow lines but clear evidence for a steady rise in variance in the

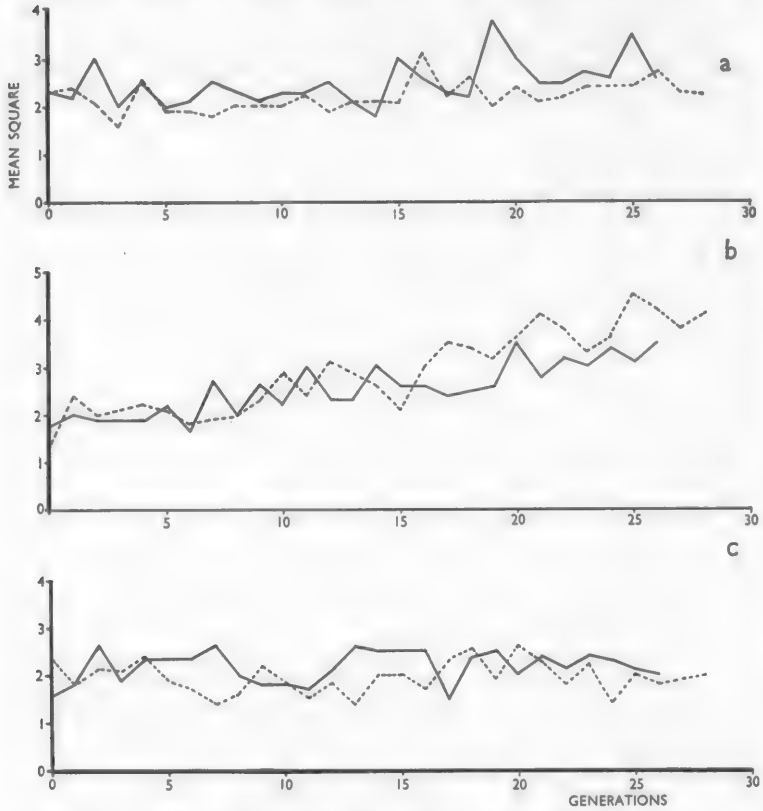


FIG. 6.—The within sex and culture mean squares. a 50 per cent., b 25 per cent., c 0 per cent. gene-flow. Broken curves : A lines. Solid curves : B lines.

25 per cent. gene-flow lines. Disruptive selection in these latter conditions can increase phenotypic variance considerably.

Heritability tests were carried out at different times on the different lines and the coefficients of regression of offspring on parent mean are listed in table 2. Heritability within each half of the 25 per cent. gene-flow lines was high. There is also high heritability in the high halves of the Line 50/A, though heritability in the low halves of the 50 lines was not detectable and the high half of line 50/B has a less

TABLE 2

*Heritability tests*

| Line | Generation tested | High half | | Low half | |
|---|---|---|---|---|---|
| | | b | P | b | P |
| 0/A | 13 | 0·00 | large | −0·30 | large |
| 0/B | 19 | 0·16 | ≏0·2 | 0·23 | ≏0·02 |
| 25/A | 21 | 0·62 | <0·001 | 0·65 | <0·001 |
| 25/B | 17 | 0·52 | <0·001 | 0·35 | <0·01 |
| 50/A | 21 | 0·45 | <0·001 | 0·01 | large |
| 50/B | 17 | 0·26 | ≏0·02 | 0·11 | >0·05 |

significant regression coefficient. Heritability in the 0 per cent. gene-flow lines was negligible, except for the low half of 0/B, where it is significant, the reasons for which will appear later.

### (iii) *Asymmetry and fertility*

The regression coefficients of bilateral asymmetry of sternopleural chaeta number on generations are listed in table 3. The measure of asymmetry used was the sum of the difference between sides, sign ignored, divided by total chaeta number (A/T), a transformation that to some extent compensates for the correlation in the Dronfield stock

TABLE 3

*Regressions of A/T on generations*

| | b | P |
|---|---|---|
| 0/A | 0·20 | >0·05 |
| 0/B | 0·04 | large |
| 25/A | 0·56 | <0·001 |
| 25/B | −0·04 | large |
| 50/A | 0·42 | <0·001 |
| 50/B | 0·15 | <0·001 |

of asymmetry with mean. It has been suggested by Reeve (1960) that A/T-6 would be a better transformation. However, as A/T has some slight empirical justification (Thoday, 1958*b*), but A/T-6 is based solely on intuitive "knowledge" of the true physiological relations in flies, we prefer A/T. The regressions are calculated using A/T × 1000 for convenience.

All the regressions are positive except that for 25/B which is negligible, the joint regression is significant, and there is significant variation of the coefficients. It is notable that the significant responses are all in the disruptive selection lines. These figures suggest that disruptive selection can pick out developmentally unstable genotypes though it does not always do so.

Fertilities, as measured by the number of flies produced per culture, were also assessed in the lines. They declined significantly with generations of selection, but as there were no significant differences between the regression coefficients of the different lines they are relatively uninformative.

### (iv) *Chromosome assays*

At generation 25 or 27 the selection experiment was ended, and genomes were extracted from the lines for chromosome assay.

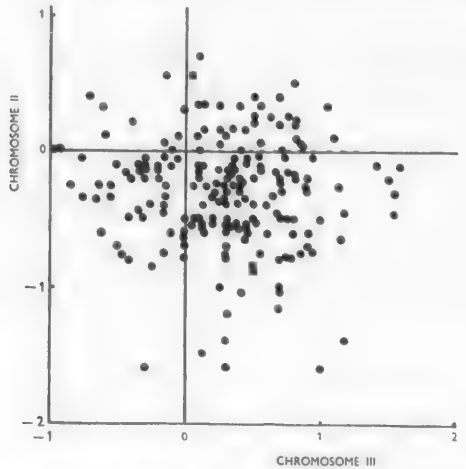


FIG. 7.—The results of assaying the Dronfield wild stock against *y bw st*. Differences o chaeta number between +/*bw* and *bw*/*bw* flies are plotted as second chromosome effects : differences between +/*st* and *st*/*st* are plotted as third chromosome effects. This fig. is plotted on the same scale as figs. 8-13.

The genomes were extracted using a newly made *ŷy bw*/*bw st*/*st* stock and the *y bw*/*bw st*/*st* inbred stock that was used for similar assays by Thoday and Boam (1959).

It was decided to extract genomes from all the flies that would have been used to maintain the selection lines had they been continued. The flies were selected as if for continuance of the lines and by mating males to the attached X stock, and females to the *y bw st* stock, and selecting ten $F_1$ males from each fly's progeny, ten genomes were preserved from each of them. Chromosome IV as was ignored.

Each of the 480 $F_1$ males was mated to a *ŷy bw*/*bw st*/*st* female and the resulting 480 genomes were preserved by repeated crossing to females of the attached-X stock. In due course the genomes were assayed by counting five flies of each sex and each eye colour. It was

not, of course, possible to grow all the assay cultures together. The assays were done in six batches of 80. No overall difference between the batches emerged so that we may treat them together.

Differences of sex difference between assays of different genomes detect variation of X chromosomes. The markers *bw* and *st* enable effects of Chromosomes II and III to be assessed. The results showed little effect that could be associated with Chromosome I (X). The effects of Chromosomes II and III are illustrated in figs. 8-13, in which the differences between $+/bw$ and $bw/bw$ flies are plotted as effects of Chromosome II, and the differences between $+/st$ and $st/st$ flies are plotted as effects of Chromosome III, as was done by Thoday and Boam (1959, fig. 3). Fig. 7 shows the results of a comparable assay of genomes taken from Dronfield stock females for comparison. We are indebted to Mr Gibson for permission to use his data.

It should be stressed that Mr Gibson's assay was made with the $y/y\ bw/bw\ st/st$ inbred stock that was used by Thoday and Boam (1959), whereas the present assays were made against the $\hat{yy}\ bw/bw\ st/st$ stock which had been newly bred for the purpose, since it seemed necessary to make preservation of X chromosomes as well as the two large autosomes possible. The two assays are not therefore strictly comparable. In particular the attached X stock, being newly synthesised, was more variable than the inbred stock, so that results may be blurred by significant variation of the marker chromosomes with whose effects the effects on chaeta number of the extracted chromosomes are compared.

i. *The o lines* The assays of Line o/A (fig. 8) show clearly that both Chromosome II and Chromosome III contribute to the difference between the high and low halves of this line. Comparison of this assay with that for the Dronfield stock suggests that there are more extreme low third chromosomes and high second chromosomes in the o/A line, and that there is more association between high seconds and thirds and between low seconds and thirds. That the high half has the most effective new chromosomes is consistent with the behaviour of the means illustrated in fig. 1.

The assay of o/B is strikingly different. The low half of o/B responded more than the low half of o/A (figs. 1 and 2), and this was clearly the cause of the greater divergence of o/B. The assay shows that this was due to the establishment, in the low half of o/B, of new second chromosomes of extreme low chaeta number effect. It further shows that this low line, despite its small population size, was heterozygous for these low second chromosomes. Such low second chromosomes have not been found in the Dronfield wild stock, and seem likely to be recombinant products of Dronfield chromosomes. Their heterozygosity in the low half of o/B explains the heritability results given on p. 205, and seems to imply that they must be chromosomes that give flies of low fitness when homozygous. These low chromosomes are reminiscent of the recombinational lethals shown by Gibson and Thoday

(1960) to be responsible for the low chaeta number flies in the polymorphic population produced by Thoday and Boam (1959). They are, however, not the same. Mr Gibson has tested them, and they are not homozygous lethal in his test conditions, and they are not lethal

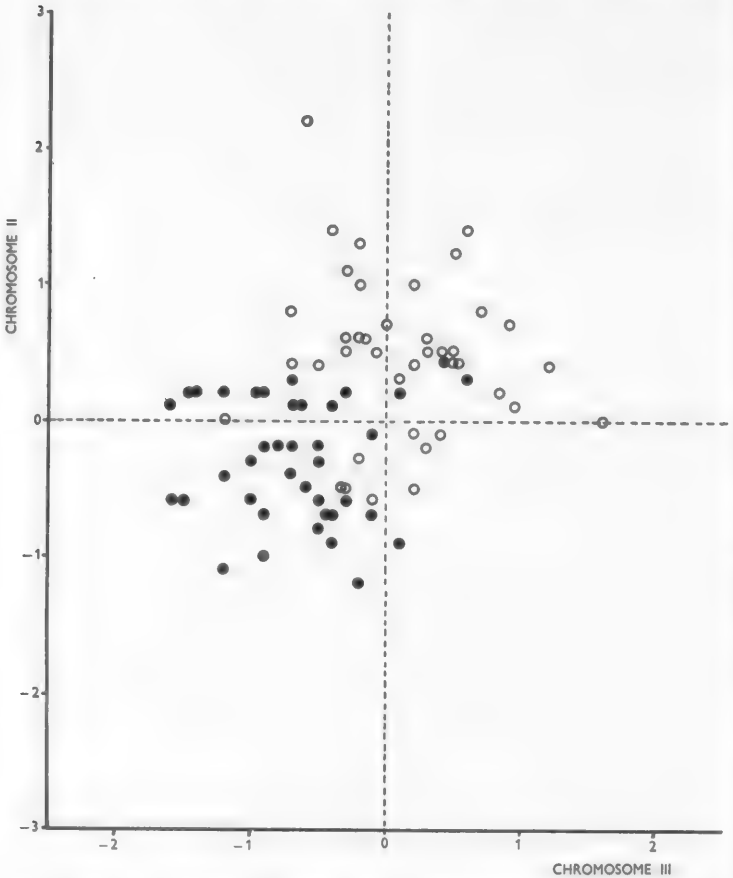


FIG. 8.—The assay results for o/A. Open circles: genomes from the high half of the line: solid circles: genomes from the low half.

when combined with his recombinational lethals. Further tests of these chromosomes are in hand. They may prove to give sterility which would explain the heterozygosity of the line.

The assay also shows that high second chromosomes were produced in the high half of o/B.

ii. *The 25 lines.* 25/A like o/A responded mostly on the high chaeta

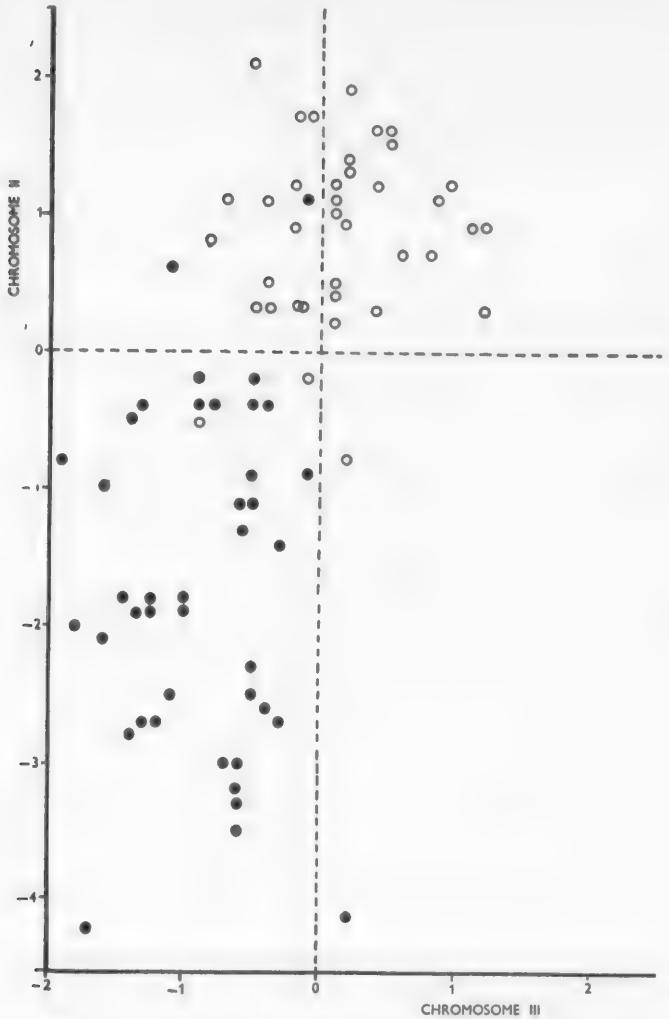


FIG. 9.—The assay results for o/B. Open circles: genomes from the high half of the line: solid circles: genomes from the low half.

O

number side (fig. 3). The assay results (fig. 10) are similar in kind to those for 0/A, but the responses have clearly involved both the second and the third chromosomes. There is greater variation in the assay

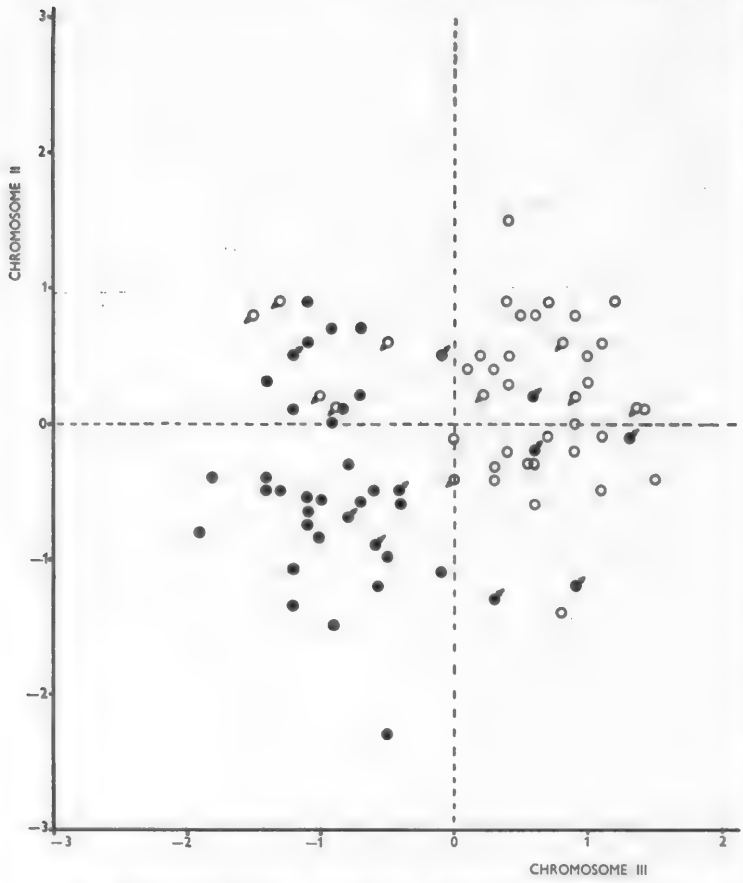


FIG. 10.—The assay results for 25/A. Open circles: genomes from the high half of the line: solid circles: genomes from the low half. The genomes from flies responsible for the gene-flow in the line are marked with arrows showing the direction of migration.

data for 25/A than in that for 0/A, and there is less overlap between the non-migrant genomes than there is between the genomes of the two halves of 0/A. We conclude that though 0/A and 25/A both diverged (fig. 5), the disruptive selection involved in 25/A produced a greater range of chromosome types than did the directional selection in 0/A.

The range is also greater than in the Dronfield stock. It is as if the gene-flow in 25/A permitted a transfer of genes to the appropriate half of the population which could not occur with complete isolation.

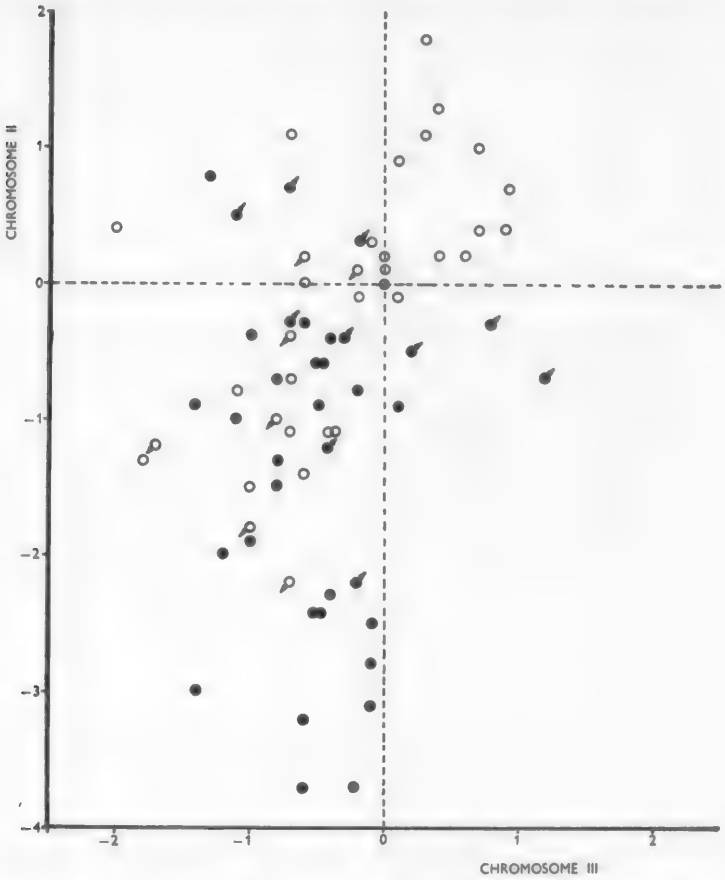


FIG. 11.—The assay results for 25/B. Open circles: genomes from the high half of the line: solid circles: genomes from the low half. The genomes from flies responsible for gene-flow in the line are marked with arrows showing the direction of migration.

With complete isolation, bristle decreasing genes homozygous in the high half, and bristle increasing alleles homozygous in the low half could limit divergence. With some gene-flow these genes could be transferred and exploited. We thus see the possibility that isolation may sometimes be a factor *limiting* divergence, as suggested by Thoday (1953, 1958*c*).

The assay of 25/B genomes (fig. 11) is quite different, and rather like the assay of 0/B (fig. 9). There was some response in Chromosome III but 25/B responded largely in Chromosome II, and its low half is clearly heterozygous for a powerful second chromosome effect.

These low second chromosomes have been tested by Mr Gibson,

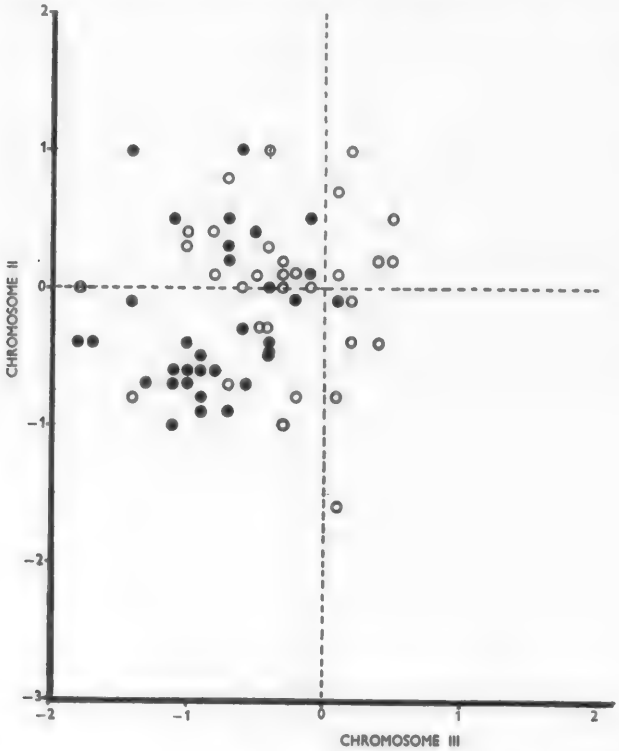


FIG. 12.—The assay results for 50/A. Open circles: genomes from the high half of the line: solid circles: genomes from the low half.

and prove homozygous viable and different from the low second chromosomes of the original polymorphic population of Thoday and Boam (1959), though it is not yet known whether they are the same as the low second chromosomes in 0/B. It is clear and striking that 25/A and 25/B achieved the same order of divergence by different genetical means, and that neither of these divergences had the same cause as that in the original population of Thoday and Boam.

iii. *The 50 lines.* The assays of the 50 lines are less informative, which is not surprising in view of the slight and variable divergence

achieved in these lines. However, comparison of these assays (figs. 12 and 13) with that for the Dronfield wild stock, show clearly that disruptive selection has increased the variety of second chromosomes. There are higher chaeta number second chromosomes in both assays, and lower second chromosomes in 50/B than in the wild stock. The

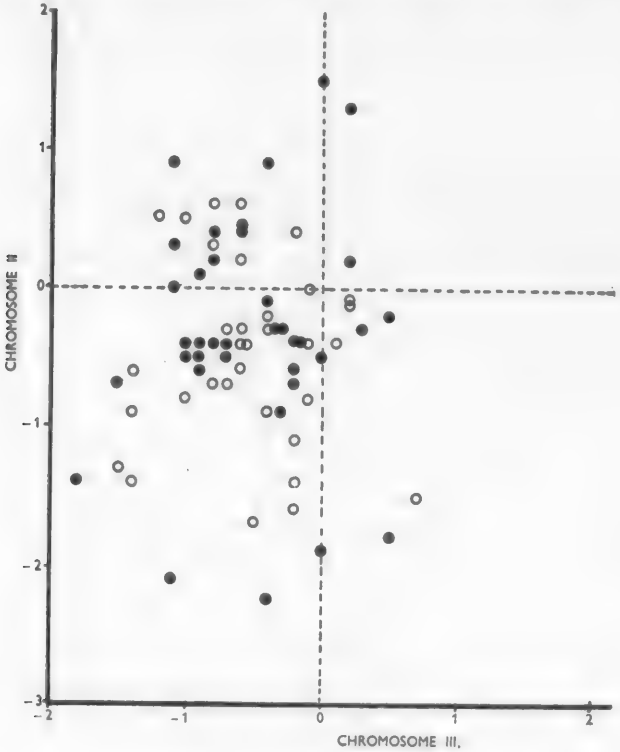


FIG. 13.—The assay results for 50/B. Open circles: genomes from the high half of the line: solid circles: genomes from the low half.

range of third chromosome effects is 2·3 chaetæ in each assay which is also the range in the Dronfield assay, so that there is no evidence that new third chromosomes were produced in this line.

It is to be noted that 50/B shows clear signs of producing low second chromosomes, but that 50/A does not. 50/B originated from the same foundation cultures (Z) as 0/A which showed no sign of such extreme low second chromosomes. We thus have three populations 0/B (from Y), 25/B (from X) and 50/B (from Z), all from different foundation cultures all producing extreme low second chromosomes such as

have never been found in the Dronfield stock. These facts strengthen the view that the origin of such extreme chromosomes is recombinational rather than mutational (see also Gibson and Thoday, 1960). We know that the 0/B and 25/B low second chromosomes differ from the low second chromosomes in Thoday and Boam's (1959) $D^+$ population. It is almost certain that the 50/B low second chromosomes are different for they give less extreme chaeta numbers. It seems as if the Dronfield wild stock can produce recombinant second chromosomes in several ways.

## 4. DISCUSSION

The results reported here leave no doubt that disruptive selection is capable of bringing about divergence of high and low components in a population despite 25 per cent. gene-flow between the two. The result is a polymorphism (see fig. 14). We expected this in view of the

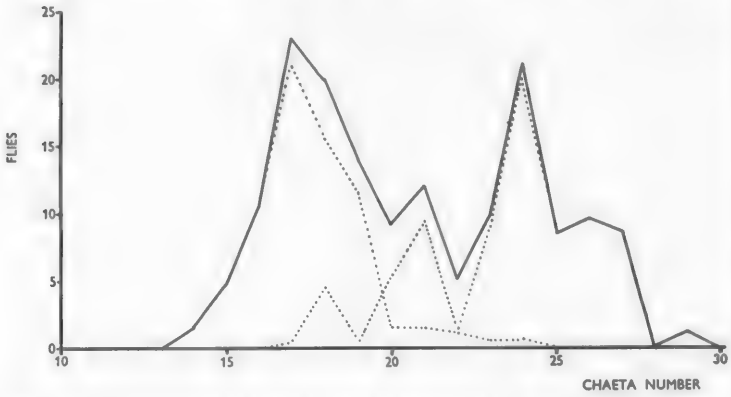


FIG. 14.—The distribution of chaeta number in generation 25 of line 25/A. The dotted lines continue the distributions of the halves of the populations.

results of Thoday and Boam (1959) who obtained divergence with 50 per cent. gene-flow. As we also expected, the divergence achieved with 25 per cent. gene-flow is greater than that in Thoday and Boam's population. Their divergence was 3 chaetæ per fly, after 37 generations, whereas the two 25 per cent. gene-flow populations had differences of 5 chaetæ per fly.

The present divergences were the result of heterogeneity of both second and third chromosomes, but it does not seem that either of the present lines produced more extreme chromosomes than were produced in Thoday and Boam's $D^+$ line. The $D^+$ low chromosome II gave the same order of chaeta number as that in 25/B, but the $D^+$ high Chromosome III was more effective than any high chromosome in

the present lines. The greater divergence obtained in the present lines, does not, then, arise from the production of more diverse chromosomes. In fact the diversity of chromosomes obtained from Line 25/A is considerably less than that in the $D^+$ line though the difference between high and low halves of 25/A is nearly twice as great as in $D^+$. The greater divergence was perhaps possible because the 25 per cent. gene-flow system permits the selection of extreme chromosomes in homozygous form. It is interesting to note in this connection, that the extreme low second chromosomes in the $D^+$ line were homozygous lethal (Gibson and Thoday, 1959) whereas those in 25/B are not (Gibson, unpublished).

It is of considerable importance that the present assays, together with the results of Gibson's tests which demonstrate the extreme low Chromosome II in 25/B to be different from that in the $D^+$ line, show that none of the three lines, $D^+$, 25/A or 25/B has responded to disruptive selection in the same way. This makes it unlikely that the Dronfield stock is peculiar in possessing a few heterozygous loci from which a polymorphism can readily be built up by selection. On the contrary, it is now clear that there are many ways in which this population can respond to disruptive selection despite its initiation from one wild female and her mate(s), and subsequent inbreeding in the laboratory. This is a nice demonstration of the remarkable genetic versatility of a wild stock of an outbreeding species.

The most striking fact about the responses in the two 25 per cent. gene-flow lines is their magnitude relative to the responses in the 0 per cent. gene-flow lines. The divergence of the two completely isolated halves of the two 0 per cent. gene-flow lines, was no doubt limited by the very small population size involved, and with larger population sizes we would expect isolation to permit greater divergence than 25 per cent. gene-flow. Nevertheless, the results suggest that in a heterogeneous habitat there will be plenty of scope for the divergence of an appropriate range of ecotypes despite random mating between them, as suggested by Thoday and Boam (1959), and Thoday (1960).

It is possible to object to this conclusion on the grounds that the present 25 per cent. gene-flow lines are maintained by a positive assortative mating system. To test the cogency of this objection we have taken a sub-line from Line 25/B and maintained it under disruptive selection with negative assortative mating. The mating system was exactly as in table 1, with the exception that the males taken from cultures A and B for mating with females of cultures C and D were selected for high chaeta number and the males taken from cultures C and D for mating with females of A and B were selected for low chaeta number. The results are illustrated in fig. 5. The change in regime at first reduced the difference between the high and low halves of the population, but recovery was rapid, and the demonstration that disruptive selection can maintain a difference

even with 25 per cent. gene-flow and negative assortative mating is clear. It seems reasonable to conclude that disruptive selection could produce and maintain considerable deviation under random mating.

## 5. SUMMARY

1. Two populations of *Drosophila melanogaster* have been exposed to disruptive selection with positive assortative mating and 25 per cent. gene-flow between the half selected for high and the half selected for low sternopleural chaeta number.

2. Two lines were run with 0 per cent. gene-flow between their halves, and two with 50 per cent. gene-flow. All the six lines were established from the same wild stock " Dronfield ".

3. Divergence was greatly limited by 50 per cent. gene-flow, but with 25 per cent. gene-flow it was, though slower, ultimately of as great magnitude as with complete isolation (0 per cent. gene-flow).

4. Chromosome assays showed the disruptive selection lines to contain a greater variety of chromosomes than the Dronfield wild stock. The two 25 per cent. gene-flow lines had responded in different ways for one contained extreme chaeta number second chromosomes, the other did not.

5. One of the 0 per cent. gene-flow lines was heterozygous for extreme low second chromosomes in its low half. This and the similar second chromosome in the 25 per cent. line may prove to be genetically the same, but they are certainly genetically different from the extreme low second chromosomes in the polymorphic population $D^+$ established by Thoday and Boam from the Dronfield stock. In addition one of the 50 per cent. gene-flow lines contained less extreme low second chromosomes. No such chromosomes have been found in the Dronfield stock.

6. The $D^+$ low second chromosome is known to be a recombinant product of other second chromosomes in the stock, and evidence points to these new ones also being recombinational in origin. There seem to be several ways in which this stock may respond to selection to produce polymorphisms.

7. The magnitude of the divergences in the 25 per cent. gene-flow lines is strong evidence that random mating cannot prevent divergence under disruptive selection. This conclusion is given force by the maintenance of most of the difference between the low and high halves of one 25 per cent. gene-flow population when put under a similar regime with negative assortative mating.

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# EFFECTS OF DISRUPTIVE SELECTION

## V. QUASI-RANDOM MATING

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Received 8.iii.61

## 1. INTRODUCTION

THODAY (1958, 1959, 1960), Thoday and Boam (1959) and Millicent and Thoday (1961) have shown that disruptive selection can increase the variety of genotypes in a population. All their experiments were done with rather special systems involving either negative or positive assortative mating between the selected phenotypes. It therefore seemed worth while to run a brief pilot experiment to see what degree of variance could be maintained with a system simulating random mating, which (Thoday and Boam, 1959) is not to be confused with panmixia. Such a system was devised and an experiment started. It had to be stopped when the senior author moved to Cambridge, but the results seem sufficiently interesting to be worth reporting briefly.

## 2. METHODS

The character used was sternopleural chaeta number in *Drosophila melanogaster*. The system devised to give quasi-random mating is simple. It involved selecting from a population a number of flies of each sex (8) with high and the same number with low chaeta number. The resulting four groups of flies were each divided at random into two lots of four flies each. They were then set up for 24 hours in four mating tubes. The first contained four high females with four high males. The second contained four high females with four low males. The third contained four low females with four high males, and the last contained four low females with four low males. Twenty-four hours later the flies were removed from the mating tubes and the males were discarded. The sixteen females were then placed together in a single culture bottle for 72 hours. The progeny were collected as virgins and forty of each sex were assayed for chaeta number, and the selection and mating process was repeated. The degree to which this system simulates random mating will depend of course upon the degree to which the four different classes of mating produce equal numbers of offspring. The system could easily be arranged so as to ensure that equal numbers were included in the sample assayed by placing the four groups of females in separate cultures and taking equal numbers of progeny from the four cultures. However, the authors were at the time by themselves already maintaining too many selection lines, and time, particularly for virgin collecting, was limiting, so that it was decided to use the easier system for the present pilot experiment. As will be seen, the evidence suggests that, except in the first generation, the system produced adequate results.

## 3. MATERIAL

The material used was the same as that used by Thoday (1960), namely the high sternopleural chaeta number line homozygous for *vg* (vg 6 of Thoday and Boam, 1961), and the low chaeta number stock homozygous for *se cp* and *e*. The experiment

was started by setting up the four possible matings between vg 6 and the *se cp e* stock as described above.

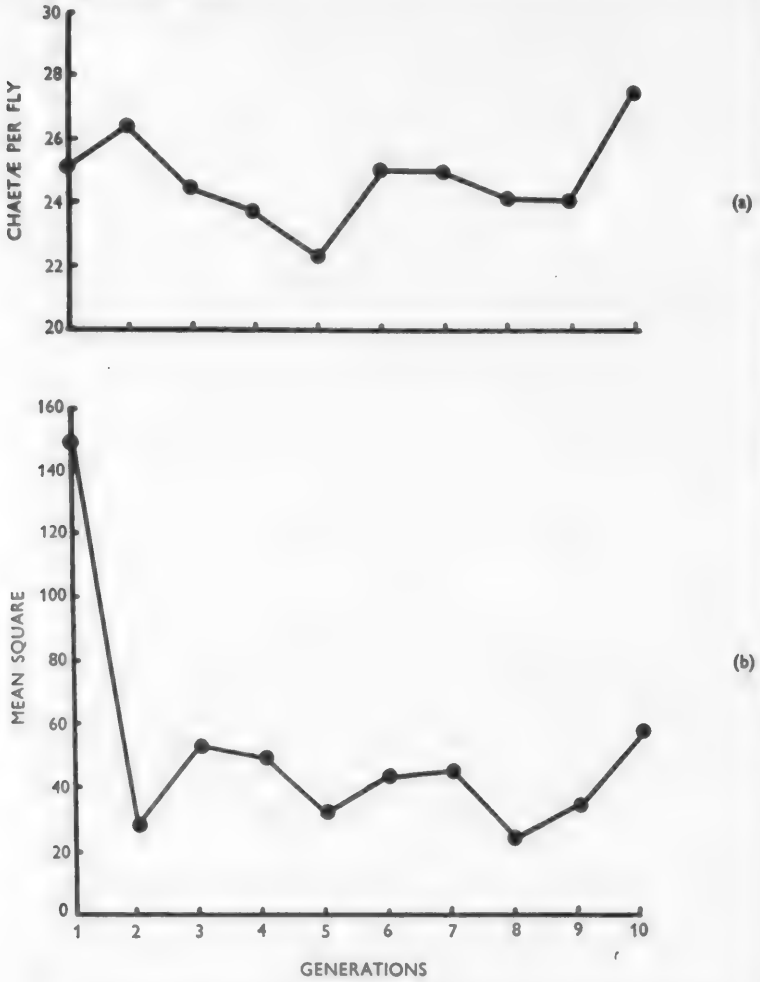


FIG. 1.—Means (*a*) and Mean Squares (*b*) for sternopleural chaeta number in each generation.

## 4. RESULTS

### (1) *Means and variances*

The means and variances of chaeta number are illustrated in fig. 1. The mean remained around 25 chaetæ which is rather below the mid value between the vg6 and *se cp e* means which were 42 and 16 respectively

when the experiment was started. The variance was very high in the initial generation but fell straight away to about 40, round which value it fluctuated thereafter. That the mean is lower than the mid parent value is explained by the fact that, in the foundation generation, the vg6 flies were less successful than *se cp e* flies. The very high variance in the foundation generation is caused by a deficiency of hybrid flies in this generation.

The mean variance from generation 2 to the end of the experiment is 41, and is higher than in the $F_2$ of the cross between vg6 and the *se cp e* stock described by Thoday (1960), which was 30. Disruptive selection with negative assortative mating in Thoday's (1960) lines derived from that $F_2$ stabilised this variance at about 15, whereas in his stabilising selection line variance was reduced to 5.

The maintenance of variance in the present line at so high a value clearly suggests that under the present system disruptive selection can be very effective. It may well be, indeed, that disruptive selection

TABLE 1

*The number of* vg/vg *and of* se/se *flies* (*80 flies assayed per generation*)

| Generation | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *se/se* | 38 | 24 | 14 | 22 | 33 | 18 | 13 | 3 | 12 | 12 | 150 |
| *vg/vg* | 22 | 14 | 17 | 19 | 22 | 16 | 14 | 13 | 2 | 5 | 128 |

will prove more effective with random mating, for the selection pressures used in this experiment are very much lower than in our earlier work. Twenty per cent. of the flies assayed were selected in the present experiment, whereas all the earlier work reported in this series of papers has involved selecting only 5 per cent. of the flies in each generation.

### (iii) *The marker genes*

It is known that two powerful chaeta number factors distinguishing vg6 and the *se cp e* stock are linked to *se* and that interacting factors are present on Chromosome II (Thoday, 1960; and unpublished), though it is not yet known how closely linked the interacting factors are to the locus *vg*. It is therefore of interest to follow the behaviour of the loci *vg* and *se* in the present line. The numbers of *vg* homozygotes and of *se* homozygotes in each generation are listed in table 1. $\chi^2$s have been calculated for the data, excluding the foundation generation, on the basis of expectation of one quarter of the population being homozygous for a mutant marker. For *vg*, the overall $\chi^2_{(1)}$ is 20·0296 showing that *vg* homozygotes form significantly less than a quarter of the population, but the heterogeneity $\chi^2_{(7)}$ is 5·0371 ($P = 0{\cdot}7-0{\cdot}5$), showing that the frequency of *vg* has not changed significantly over the generations, though the later generations seem to show *vg/vg* in lower

frequency. For *se* the overall $\chi^2$ is 6·2296 and there is therefore significant, but much less, deviation from one quarter. The heterogeneity $\chi^2$ is 46·3333, showing significant variation in proportion of *se*/*se* flies between generations, and there is some evidence of a trend in *se*/*se* frequency.

These data serve two purposes. In the first place they suggest that in this experiment, as in the experiment of Thoday (1960), disruptive selection helped to maintain the frequencies of the marker genes *vg* and *se*. *vg* frequencies can be very rapidly reduced by natural selection. Dinsley and Thoday (1961), for example, ran 6 populations heterozygous for *vg* and by the tenth generation natural selection had completely eliminated the *vg* gene from all of them.

Second they show that, since *vg*/*vg* and *se*/*se* flies together comprise less than half the flies in the population, more than half were hybrid genotypes, and hence that the extremely high variance maintained was not due to hybrid inviability or infertility of hybrid crosses despite the deficiency of hybrids in the first generation. Hence it is not unreasonable to apply the term quasi-random mating to the population.

## 5. CONCLUSIONS

It would be unwise to attach too much significance to this experiment. There was only one small line, it was only maintained for ten generations, and there were no stabilising selection or natural selection controls.

Nevertheless the variance maintained in the population was so large that it seems fair to regard the experiment as supporting the conclusion, suggested by Thoday and Boam (1959) and Millicent and Thoday (1961), that random mating would not prevent disruptive selection from maintaining (or even establishing) a wide variety of genotypes in a population. This conclusion is the more justifiable since the proportion of flies selected in this experiment (20 per cent.) is so much higher than the 5 per cent. selected in previous experiments on disruptive selection.

## 6. SUMMARY

1. A system is described for simulating random mating in a population under disruptive selection.

2. A system approaching this was used in a pilot experiment involving sternopleural chaeta number.

3. Though the selection pressure was low, so high a variance was maintained that it seems likely that disruptive selection will prove to have powerful effects despite random mating.

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# NOTES AND COMMENTS

## THE COMPLEX TRAIT AS A GEOMETRIC CONSTRUCT

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Received 1.iii.61

> ". . . I have studied the inheritance of a large number of characters which might be expected to show heterosis from *a priori* reasoning, but which do not show it. They are characters determined by a small number of genes and affecting particular organ systems "—E. M. East, 1936.

If a complex trait is the result of multiplicative gene action, it has a geometry. There is good reason to believe that such geometries are widespread. They are apparent in small grain where yield has been treated as the volume of a rectangular parallelepiped with edges X, Y and Z equal to number of heads per unit area, number of kernels per head, and average kernel weight, respectively (Grafius, 1956, 1959). Other geometries have been described for yield in corn (Grafius, 1959) and may be inferred from the classic work of Powers (1952) on yield in tomatoes and in the recent work of Williams (1959) also on tomatoes. This geometric attribute is not confined to plants, since the work of Gowen (1952) clearly demonstrates the possibility in the case of egg production in *Drosophila*.

Similarly Jinks (1955) has found evidence of non-allelic interactions which are quite probably of a geometric nature. He reports non-allelic interaction for height in *Nicotiana rustica*, and for yield in corn on the basis of the published data of Kinman and Sprague (1945), Nilsson-Leisson (1927) and Stringfield (Hull, 1946) and for yield in flax, Carnahan (1947). Jinks makes this penetrating observation, " Wherever we find evidence of overdominance we also find non-allelic interaction ".

In all of the cases that Jinks reported where non-allelic interaction occurred it was associated with a complex trait such as yield or height. On the other hand, the cases where he found no evidence of non-allelic interaction involved less complex traits such as flowering time, weight of individual fruits or shape of fruit.

The inference is clear. This multiplicative interaction could well be the result of the geometry of the complex trait. This is not to say that traits such as fruit weight do not have components, but only that with our present precision of measurement there is no evidence of multiplicative interaction.

A question arises regarding the apparent tacit assumption that all multiplicative interaction is between the components or edges of the geometric image and not at the locus level as in the classic sense. The multiplicative action need not be all one or the other, but for complex traits such as yield the apparent effect is not inter locus but between the edges of the geometric figure. In point of fact inter locus multiplication in the

* Contribution from the Michigan Agricultural Experiment Station, Journal Article No. 2545.

classic sense may lack the explosive effect necessary to explain the massive quantitative increase exhibited by complex traits such as yield of an $F_1$ hybrid in corn.

Starting with a simple system, let the various $A_1 \ldots A_n$ loci interact with the various $B_1 \ldots B_n$ loci in pairs and with summation of effects between pairs, *e.g.* $A_1B_1 + A_2B_2 + \ldots + A_nB_n$ where the various $A_i$ and $B_i$ represent loci, not alleles. As a contrasting model let the sum of all the $A_i$ effects interact with the sum of all $B_i$ effects. Then it is apparent that

$$\sum_{i=1}^{n} A_iB_i < \sum_{i=1}^{n} A_i \sum_{i=1}^{n} B_i. \quad [1]$$

Hence epistasis in the classic sense, as shown on the left of the inequality, is a much less potent hypothesis. It could be argued that there may be other possible types of inter locus interaction. For example

$$A_1 \times A_2 \times A_3 \times \ldots \times A_n \times B_1 \times B_2 \times B_3 \ldots \times B_n,$$

which under some conditions could greatly exceed the right hand side of [1]. One can only state that such a system would be extremely vulnerable to a locus failure and also that there is no evidence for such a system, while both types in [1] have been demonstrated for a wide variety of organisms. Other systems could include the combination of both sides of [1], or the combination of both additive and multiplication gene action, and so on. Gene action in a complex trait need not be restricted to any one type but it is proposed that major heterotic effects will be associated with the right hand side of [1].

For obvious reasons the classic type of epistasis will be designated " geometric-additive ", while the right hand side of [1] will be called " additive-geometric ".

As it now stands the additive-geometric model permits the restoration of the dominance hypothesis to a position of eminence. No satisfactory explanation has ever been advanced to refute the crucial* experiment of Richey (1931) except to show that dominance *per se* could not account for the degree of heterosis found in many instances (East, 1936 ; Hull, 1946 ; Crow, 1952) the inference being that heterosis must be due to a physiological stimulus between alleles. Admittedly, intra-allelic interactions have been demonstrated for simply inherited traits (Quinly and Karper, 1948) but in so far as the complex trait is concerned the additive-geometric concept appears, in combination with relatively small amounts of dominance for each edge, to offer a more logical explanation for the heterosis puzzle. For it should be evident that relatively small amounts of dominance at each edge could, when multiplied together, readily account for $F_1$ vigour in a complex trait.

An attempt has been made to illustrate the usefulness of a geometric concept in predicting components of complex traits due to multiplicative gene action (table 1). The multiplicative gene action must result in areas, volumes or higher dimensional products, otherwise the products are nonsense. Some of these values are exact, as for example yield in small grain where the product of the three components is identically yield. In this case

* The idea of the convergent improvement experiment being crucial was borrowed from W. H. Leonard.

TABLE 1

*Geometrics of complex traits*

| Trait | Postulated components |
|---|---|
| Height | Number of internodes × average length of internode or alternately, average cell length × average number of cells on the long axis of the plant |
| Leaf shape as a ratio of length/width | Length × 1/width or alternately, the product of length and 1/width as functions of cell diameters and average number of cells on the long and short axis |
| Total leaf area per plant | Number of leaves × average leaf area |
| Yield * | |
| Small grain | Heads/unit area × kernels/head × av. kernel weight |
| Corn † | Ears/plant × rows/ear × kernels/row × average kernel weight |
| Tomatoes † | Weight/fruit × fruit/plant or alternately, weight/locule × locules/fruit × fruit/plant |
| Production of eggs | |
| Lifetime egg *number* | Length of egg laying period × average daily egg production |
| Lifetime egg *weight* | Length of egg laying period × average daily egg production × average egg weight |
| Lodging resistance in small grain | Force the culm is capable of supporting × 1/height |

* Note that the geometry here is exact in most instances.
† Assuming constant stand.

the trait is a mental construct and therefore the relationship should be exact. In others, *e.g.* the components of a trait such as kernel weight, it will only approximate the true kernel weight since the kernel is only approximately ellipsoidal.

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## THE FUTURE OF MAN: A REPLY

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Received 5.iv.61

Professor Darlington's review of my broadcast lectures on *The Future of Man* appears in the December 1960 issue of *Heredity*. In the main, he construes the lectures as an attack upon a " bogeyman ", an " enemy ", an " unidentified antagonist " who practises what I have chosen to call " geneticism ". The length and style of his review, its misrepresentations, and its agitated appeals to an unseen audience (Darlington asks no less than twenty rhetorical questions) combine to suggest that he has identified my unknown antagonist with himself.

Of my first lecture Darlington says that I advocate cohort analysis because " it is likely to succeed where other methods have failed in predicting the future numbers of our population ". The point of the first lecture has therefore escaped him. What I actually said was that " predictions founded upon cohort analysis are somewhat more exact in the sense that one can foresee a little more clearly what follows from one's assumptions; and if these predictions are wrong, as to some extent they surely will be, it will be easier in retrospect to see which assumptions were faulty and which factors changed in unforseeable ways ".

Darlington reviews my second lecture at great length. His philosophic reflections upon it have, for me, a certain self-taught quality that make them hard to follow, but he is particularly contemptuous of my saying that " it is impossible, indeed self-contradictory, that an animal should have evolved into the possession of some complex and nicely balanced genetic make-up which rendered it unfit ". This statement is true, and the term " self-contradictory " is to be taken in its strictest sense. Darlington apparently deplores the ambition to cure phenylketonuria, for if we achieve it, " shall we not in some sense be arranging for a particular type of hereditary imbecile to breed ? " As he does not answer the question, I shall do so for him : No. Darlington must distinguish between the genetic singularity and its somatic manifestations of the first or second order. He writes as if he thought the genes themselves were mentally deficient. Potential victims of phenylketonuria whose metabolic disorder has been circumvented will still suffer

from a genetic disability, for they cannot eat what they choose. It is a restriction of freedom, then; certainly worse than being obliged to wear spectacles, but not so much worse that we should be content to see them die. There is much to be said for trying to prevent the genetic conjunction that leads to phenylketonuria in the first place (for example, by identifying the heterozygotes and dissuading them from marrying each other), but it should be realised that such a policy will allow the offending gene to increase in the population unopposed.

I forbear from comment upon Darlington's homespun anthropological homily, which begins with the remarkable sentence "Primitive societies are made up of men and women who are able to do everything they need for their own survival and propagation".

In studying my third and fourth lectures, Darlington finds me to have said that "we must not be sure . . . of any genetic conclusion". He himself is confident of his ability to control evolution in man, in the sense of directing it towards a predetermined goal. Some of the principles that would underlie his attempt to do so are made clear by his views on the genetic consequences of birth control. Darlington attributes the fall of the birth rate, and its subsequent rise, to genetic agencies:

> "Previously children had been born to parents merely in accordance with their ability to beget and bear them. Now they were born to parents in accordance with their willingness. Thus, for the first time in evolution, parents who did not want children, or want so many of them, were selectively disfavoured. Conversely a selection began which favoured specifically the property of wanting children. We should therefore expect a sag in the birth rate; and after the sag a recovery. As indeed seems to have happened."

"For Professor Medawar this would be perhaps a worthless speculation". I fear so. "A piece of geneticism, he might say." I do. The facts are that in this country the average size of completed families fell smoothly from about six for couples married in the 1860's to about three for couples married in 1910, and then to about two for couples married in the 1920's. Furthermore, cohort analysis has shown that a large fraction of the specially numerous births that occurred towards the end of and shortly after the war can be attributed to the decision of married couples to have *then* the children they were disinclined to have during the dark days of the war itself. To attribute all these rapid secular changes to the action of natural selection upon inborn differences in the inclination to have children is, in my opinion, ludicrous.

I reaffirm my contention that the discovery of the conservation of genetic variance that is made possible by the Mendelian system of heredity was a discovery of Newtonian stature. For some reason Darlington feels that the concepts of stabilising selection, of the effects of disuse and of the stability of chromosome numbers all derogate from the importance of this discovery. They do not.

My fifth lecture dealt with the possibility of a secular decline of intelligence. The rational case for a decline of intelligence is based upon the negative correlation between a child's score in an intelligence test and the size of the family he comes from. I said, and I repeat, that for the development of this argument, "there is no need to assume that professional men

are innately more intelligent than labourers ". The whole approach bewilders Darlington, for he takes it quite for granted that intelligence tests measure " innate " intelligence : " the intelligence tests which the lecturer accepts for his argument have indicated that professional men are innately more intelligent than labourers ". And so again he misses the point. Darlington does not realise how *difficult* these problems are, how many highly capable people have wrestled with them, how uncertain our inferences still are.

It is equally clear that Darlington has not grasped the argument of my last lecture ; indeed, he confesses himself utterly entangled in it. He speaks of an inversion of metaphors, of heredity being called instruction and instruction heredity, and of many other difficulties of his own making. This is beyond remedy from me.

Finally, Darlington takes it upon himself to speak for science and for genetics generally; " the scientist ", he tells us, will think this, " the geneticist " that. I dispute Darlington's claim to speak for all scientists and all geneticists. He speaks for himself.

# REVIEWS

**RADIATION PROTECTION AND RECOVERY. Edited by Alexander Hollaender. Pergamon Press, London. 1960. Pp. 392, with 56 figures. 70s.**

As Hollaender points out in the introduction, " this volume contains discussions of the biological, physiological, and biochemical methods that have been developed for protecting living organisms against radiation damage ". Of special interest to geneticists are the chapters on the protection and recovery of the cell by Whiting, on the modification of chromosome aberrations by Wolff, the protection and recovery mechanisms in seeds by Davidson and genetical protection against ionising radiation by Conger.

Of more general interest, but with genetic implications, are the chapters on protection and recovery in bacteria and fungi by Stapleton, protection of whole-body radiation injury with bone marrow therapy by Smith and Congdon, delayed somatic effects by Odell, Cosgrove and Upton, the effect of radiation on antibody formation by Makinodan and Gengozian, and photoreactivation by Jagger.

Factors which may modify damage induced by ionising radiation include the stage of nuclear development, mechanical disturbance, radiation intensity, ion density, water content of the cells, temperature, oxygen tension and chemical agents. These agents may act by modifying the initial chromosome breaks, or, more often, by hastening or delaying the restitution of the induced breaks in the chromosomes. Most of the chemical protective agents appear to function by reducing oxygen tension.

Protective agents which function at the cell or nuclear level also function at the organism level, as would be expected on a genetic basis. Similar reactions to the various protective agents are found in all organisms from bacteria to mammals. These similarities also extended to the macromolecular level *in vitro*, as shown by Alexander in the opening chapter.

The rapid extension of the field of radiation biology makes it very difficult to keep up with the publications in more than a limited area of research. These review monographs serve a very useful purpose in reviewing the literature and providing a general summary of work in allied fields. The authors of this monograph have done an excellent job.

KARL SAX.

**ERBLICHE SCHÄDEN DURCH IONISIERENDE STRAHLEN. By Richard Focke. VEB Georg Thieme, Leipzig. 1959. Pp. 78. DM 10.45.**

In the Atomic Age it is essential that everyone should understand the genetic hazards of ionising radiation. Even if we abolish atomic weapons the industrial use of atomic energy also presents problems. The author has dealt with these hazards in a concise and simple manner. The various types of ionising radiations and their biological effects are described. The various factors which may modify radiation sensitivity are listed briefly under physiological, environmental and genetic variation, but one of the most important factors, chromosome size, is not included.

Major attention is given to the induction and transmission of deleterious mutations induced in man by ionising radiation. Included are the types of

mutation, their genetic transmission to future generations, their deleterious and lethal effects and their persistence in human populations. The deleterious effects on domestic plants and animals are also discussed.

The hazards are great, but so also are other aspects of our modern civilisation—automobiles, air pollution and the stress of urban life. An atomic war could wipe out the human race, but the highly resistant viruses and bacteria and the plants and animals with small chromosomes would survive. Might they not provide the foundation for a new evolutionary cycle which eventually would produce a more rational human population less likely to destroy itself by its own inventions?

KARL SAX.

**RECENT ADVANCES IN HUMAN GENETICS. Edited by L. S. Penrose and Helen Lang Brown. J. A. Churchill, London. 1961. Pp. 194. 27s. 6d.**

The " Recent Advances " series so well known in medicine offer unique opportunities to authors. They need only write about subjects with which they are familiar and they may assume that their readers know at least the rudiments of their subject. Subjects can be selected where real advances have been made and an authoritative review is timely. This little volume makes good use of these advantages.

L. S. Penrose, the editor, contributes the chapter on mutation, reviewing the rationale of estimates in man and tabulating a selection of estimates. Possibly some of the paragraph headings suggest that more is known than the contents merit, as for example, the one entitled " The Incidence of Spontaneous Chromosomal Aberrations in Man ". Only for mongolism are data available which enable more than a shrewd guess at frequency to be made. There is a mistake in table 7 in the section which considers the association of paternal age with mutation. The figures for Northern Ireland are not those given by the author quoted.

Penrose has also contributed the chapter on " Genetics of Growth and Development of the Foetus " which reviews the evidence for the inherited influences on gestation time, birth weight and peri-natal mortality although most of this work is not new. He also mentions briefly some aspects of the factors related to the ætiology of congenital malformations in man. Table 19, page 68, has suffered some malformation which makes it unintelligible.

D. G. Harnden has managed to compress into twenty pages a remarkably comprehensive review of the developments of work on human chromosomes in the last few years. Some of the priorities for first reporting of abnormal karyotypes given here may be disputed by other workers but this is just the chapter to be enjoyed by those interested, even if not involved, in chromosome work.

O. J. Miller's chapter on " Developmental Sex Anomalies " is too compressed to permit of adequate clinical descriptions and perhaps tends to present an over-simplified picture of a very complex subject. " The Abnormal Hæmoglobins " by P. S. Gerald, " The Inheritance of Dermal Ridge Patterns " by Sarah B. Holt and C. A. B. Smith's contribution on statistical methods are all authoritative and stimulating.

Many human geneticists will be very grateful for Renwick's chapter on " Elucidation of Gene Order " (perhaps a rather grandiose term). Particularly useful is the discussion of the criteria of efficiency of markers and

the evaluation of those at present available. The unsuspecting may not realise the immense amount of computing required by the numerical methods described, but then they may not suspect either the hundreds of miles to travel and the night and week-end work needed in visiting families, examining patients and relatives and collecting specimens. As a rough guide, for each large family with segregating dominant traits, allow two months' work.

It is to be hoped that new editions of this book will appear not too infrequently. There is a real need for such excellent up-to-date reviews.

ALAN CARTH STEVENSON.

**DIE GESCHICHTE DES MENSCHEN. By G. H. R. Koenigswald. Springer, Berlin. 1960. Pp. 148, 88 figures. DM 8.80.**

Forty years ago the palaeontologist fitting human remains into an evolutionary series had a task with which he could deal almost alone. Stratigraphical dating was often uncertain; cultural dating was often absent. But since 1864 each find could be given a Latin name representing a species, a discontinuous entity in space and even in time. And with only one exception, *Eoanthropus*, the whole series could be arranged in unquestionable linear orders of progress and descent. To the geneticist the picture revealed by human fossils was not so satisfying. He expected continuity rather than discontinuity of variation. He expected continuity in time and sometimes even in space. He also expected evidence of hybridisation as well as of extinction. To him the simplicity of the record was merely evidence of its imperfection.

To-day the picture has changed in a genetically understandable direction. Some gaps have been filled but only to reveal far more new gaps. Now the author of this little book is Professor of Palaeontology in the University of Utrecht and is well known for his discoveries in Java. For him these results are puzzling: " beinahe jeder neue Fund gibt uns neue Rätsel auf " (p. 110).

To solve these riddles, it is clear, new methods are needed: several disciplines need to co-operate. We want general discussion. The purpose of this book, in a series of " Verständliche Wissenschaft ", might have been to propound the riddles as a basis of such discussion. This, however, Professor Koenigswald does not seriously attempt to do. For the general reader his illustrations, photographic and diagrammatic, offer an excellent documentary record of fossil human bones. But his text is a summary for the expert with an emphasis on stratigraphical detail. When he leaves the lower mammals behind, he plunges into a cataract of tricky detail which will take most readers out of their depth. This, it may be said, is no fault of the author. But in such circumstances everything depends on the author's success in discovering and explaining some bases of certainty.

What are these bases of certainty? They stand at the two ends of the argument. At the beginning the basis would be a list of important fossil finds together with a map of their distribution and a suggested chronology. The technical validity of these finds is fundamental but it must not be discussed along with the conclusions to be drawn from them. At the end the other basis would be a statement about variation among living men. To assume the existence only of an average " moderner Mensch " or

" Neger " is to waste half the story. Still worse is it to divide brain capacity in cubic centimetres by body weight in grammes and name this their " relation " without explaining what has been done (tables 13 and 14).

Professor Koenigswald's book therefore seems to fall short of its immense possibilities. But it will have served a useful purpose if it encourages some new readers to take the human fossil evidence seriously and to recognise how it must be arranged in order to be understood in all its manifold (and largely genetic) bearings.

C. D. DARLINGTON.

## BOOKS RECEIVED

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THE MEANING OF EVOLUTION. By G. G. Simpson. Yale Paperbound (Oxford). 1949 Reprint. 1961. Pp. 364. 11s. 6d.
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# GENETICAL SOCIETY OF GREAT BRITAIN

## ABSTRACTS of Papers read at the HUNDRED AND THIRTY-FIFTH MEETING of the Society held on 23rd and 24th MARCH 1961, at the SCHOOL OF AGRICULTURE, UNIVERSITY OF NOTTINGHAM

### THE POPULATION GENETICS OF "SEX-RATIO" IN *DROSOPHILA PSEUDOOBSCURA*

A. W. F. EDWARDS
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In *Drosophila pseudoobscura* the X-linked gene "sex ratio" causes males carrying it to produce few or no males but nearly twice as many females as are produced by normal males. In the absence of differential viability one might expect it to increase in a population until there were no males left, and yet equilibria, with gene frequencies up to 30 per cent., have been found in natural populations. Wallace (1948) conducted a series of experiments to determine the viabilities, and set up some population cages to follow the progress of the gene. In these cages the gene was either eliminated, or was at a very low frequency when the experiment was ended. Bennett (1958) put forward a mathematical model for a sex-linked locus which he applied to Wallace's findings, but examination of his model reveals an error which makes it inapplicable to this situation. Briefly, he confounded gametic and genotypic viabilities, a course which invalidates some of his findings. The present paper describes a more appropriate model, which has been used to study the polymorphism with the aid of an electronic digital computer. It is shown that Wallace's viabilities fit neither the natural nor his experimental populations, and new viabilities are suggested for each case.

### THE EVOLUTION OF THE DIPLOID-LIKE MEIOSIS OF POLYPLOID WHEAT

R. RILEY, V. CHAPMAN and G. KIMBER
*Plant Breeding Institute, Cambridge*

Previous work has shown that the diploid-like meiotic behaviour of the hexaploic wheat, *Triticum æstivum*, is due to the activity of chromosome V. In the absence of V, corresponding homœologous chromosomes from the different genomes can pair at meiosis. A series of hybrids between wheat and rye, deficient in turn for each chromosome of the wheat complement, has shown that no other chromosome has a similar activity. Other hybrids between an *Ægilops* species and wheat, deficient for either the complete chromosome V or its long or short arm separately, have demonstrated that the activity is confined to the long arm.

None of the diploid species, from which the chromosome might have been derived in the evolution of polyploid wheat, produces an effect on meiotic pairing like that caused by V. In appropriate hybrids, one species removes the restriction on pairing imposed by V. The other species do not remove the restriction on homœologous pairing, nor do they prevent homœologous pairing in the absence of chromosome V. Consequently the restriction of pairing to fully homologous partners depends upon functions modified by mutation subsequent to the origin of polyploidy.

## LIFETIME GROWTH AND PRODUCTIVITY OF LARGE AND SMALL STRAINS OF MICE

R. C. ROBERTS
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The lifetime growth and reproductive performance of mice selected for high and low six-week weight were studied. The experimental material comprised six groups —two large strains, two small strains, a cross between large and small strains and an unselected strain.

Strains differing in weight at six weeks attained markedly different mature weights. But two strains with closely similar mature weights differed conspicuously in their rates of growth. Unlike the other four groups, the two large strains showed a sharp decline in weight in later life, probably through the depletion of accumulated stores of fat.

Large strains, as expected, had larger litters than the small strains, but they had a much shorter reproductive life, with the consequence that the small strains eventually weaned twice as many offspring per mouse as the large strains, over their whole lifetime.

The heterosis displayed by the crossbred mice with respect to reproductive capacity was striking. Compared with the better parental strain, this group weaned three times as many offspring over their lifetime, the total weight of which at weaning time was four times as great.

## BREEDING IMPROVED MILKING COWS THROUGH ARTIFICIAL INSEMINATION

J. EDWARDS and L. K. O'CONNOR
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The widespread use of artificial insemination in England and Wales (over 2 million cattle forming 60 per cent. of the National Herd are inseminated annually, some 1·5 million of these inseminations being carried out from the 23 centres owned by the Milk Marketing Board) permits the application of scientific principles to the breeding of dairy cattle. The Milk Marketing Board's plans for breeding improved milking cows through A.I. are briefly described and some of the problems encountered are discussed.

## SEX DETERMINATION IN THE HOP, AND NEW BREEDING TECHNIQUES

R. A. NEVE
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Cytological studies of a range of dioecious and monoecious hop plants suggest that morphological sex differences are determined by the X chromosome/autosome balance, which probably controls auxin levels. The Y chromosome, however, appears to be necessary for successful pollen development.

Hop breeding would be greatly facilitated if pollen could be obtained from plants whose quality characteristics can be directly assessed. This is possible with tetraploid monœcious plants, and attempts are also being made to produce fertile male flowers on genetically female plants by chemical treatment.

## THE GENETIC CONTROL OF LEAF DEVELOPMENT IN RYEGRASS

K. J. R. EDWARDS and J. P. COOPER
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Total leaf area is an important factor determining yield. As the measurement of total leaf area is very laborious the genetic control of two of its main component

characters, individual leaf size and rate of leaf production, have been studied. Estimates of heritabilities for three varieties, Italian ryegrass and Irish and Hunsballe perennial ryegrass, were obtained from parent-progeny regressions and the analysis of full sib families. These tests showed appreciable additive genetic variation for rate of leaf production in all varieties and for leaf size in Italian and Hunsballe. Small negative genetic correlations between these two characters were found in all varieties.

Selection for high and low expression of leaf size and rate of leaf production has been continued for three generations and appreciable response has occurred in all varieties. Deviations occur from the responses expected on the basis of the first generation estimates of heritabilities and genetic correlations. In most selection lines there is a marked negative correlated response between leaf size and rate of leaf production, with the result that, while there may be appreciable response in the selected character, there is no significant corresponding improvement in yield.

## GENETIC ANALYSIS OF GROWTH IN TOMATO

A. I. KHEIRALLA and W. J. WHITTINGTON
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Genetic analysis of continuous variation has usually been carried out on characters measured at only one point of time. The results to be discussed were obtained from a diallel analysis involving five tomato varieties in which total dry weight and leaf area were determined at fortnightly intervals over ten weeks of growth. The experiment has allowed both the analysis of growth rate and of dry weight and leaf areas at varying stages of plant development. The cause of heterosis in certain crosses will also be discussed.

## SOME ASPECTS OF THE INHERITANCE OF GRAIN QUALITY IN WHEAT

J. BINGHAM
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For the purposes of genetic investigation, grain quality in *Triticum æstivum* must be considered as a complex of characters. Milling quality is principally determined by the cellular structure of the endosperm; test weight and grain size being less important. The quality of the flour, for bread or biscuit making, depends largely on its protein content and the capacity of the protein to give the dough the required physical properties. The inheritance of these components has been studied in a diallel series of crosses, comprising the varieties Holdfast, Yeoman, Cappelle Desprez, Hybrid 46 and Heine VII. The genetic systems involved showed a significant degree of dominance, and virtually complete absence of genic interaction.

In a further experiment, individual grains on $F^1$ plants were graded according to endosperm structure. The classified grains, which appeared to show a wide segregation for the character examined, were sown and grain from the resultant plants was milled experimentally. Good agreement between the two tests showed that the milling type of an individual grain on a heterozygous plant is determined by the genotype of the endosperm, rather than by that of the parent. It is suggested that other components of grain quality may be similarly determined.

## MEIOTIC NON-DISJUNCTION

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Consideration is given to the several theoretically possible modes of meiotic non-disjunction and their genetic consequences.

## THE MECHANISM OF FORMALDEHYDE-INDUCED MUTAGENESIS

T. ALDERSON
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It is established by means of a chemically defined and axenic treatment medium, that formaldehyde exhibits no mutagenic activity towards *Drosophila melanogaster* by the larval feeding method, unless adenylic acid is present in the treatment medium. A reaction product formed between formaldehyde and adenylic acid in the treatment medium is postulated as the metabolite concerned in the mediation of the mutagenic activity of formaldehyde. The principal metabolite is assumed to be an adenylate dimer, where two adenylic acid molecules are linked together through their amino-groups by a methylene bridge. The stereochemical properties of this metabolite and its consequent utilisation in deoxyribonucleic acid synthesis, together with mechanisms for its mutagenic activity are discussed.

An essential role is thus ascribed to the 6-amino-group of adenylate for the formation of the adenylate dimer, since the binding of formaldehyde by ribonucleic acid mono- or poly-nucleotides appears to be a specific function of the amino-groups of the purine and pyrimidine bases. Whilst adenylate is effective when present in the treatment medium as any of the forms adenosine-3′-phosphate, adenosine-5′-phosphate, adenosine-5′-phosphate, or deoxyadenosine-5′-phosphate, the simple replacement of the 6-amino-group of adenosine and adenosine-5′-phosphate (to give inosine and inosine-5′-phosphate) completely removes the ability of these compounds to mediate the mutagenic activity of formaldehyde. Although adenosine is as effective as the adenine ribonucleotides, adenine shows no ability to mediate formaldehyde-induced mutagenesis and is actually anti-mutagenic on a formaldehyde-containing yeast-glucose treatment medium. The patterns of utilisation of purine and pyrimidine derivatives by *Drosophila melanogaster* are discussed in relation to their ability or inability to mediate the mutagenic activity of formaldehyde.

## POLARISED REPLICATION AS A POSSIBLE CONDITION FOR RECOMBINATION

O. H. SIDDIQI
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Localised negative interference in *Aspergillus nidulans* and other microorganisms shows that recombination in one zygote is confined to small regions of the chromosome. These regions called, by Pritchard, effective pairing segments, are discontinuously distributed over the chromosomes.

Among crosses involving fourteen *paba* mutants, two by two, the distribution of recombination events in this region has been studied. The strength of negative interference on either side of the interval within which a cross-over between two *paba* mutants is selected, is asymmetrical. With increasing length of the selected interval " negative interference " on the proximal side falls rapidly while on the distal side it remains unaffected.

If, as generally believed, replication is an essential condition for recombination, the results suggest that replication is polarised. This imposes a time sequence on the occurrence of recombination events within the effectively paired region. The results are consistent with those of Rizet, though in our case the distribution of outside markers permits identification of single and multiple cross-overs.

## SOME STUDIES OF THE EFFECTS OF ULTRAVIOLET LIGHT ON RECOMBINATION IN *NEUROSPORA*

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Preliminary studies suggest that ultraviolet light may increase both crossing-over and non-reciprocal recombination in *Neurospora*.

Recombination, as detected by random spore and tetrad analyses, was studied in crosses involving a pair of multi-marked stocks of *Neurospora crassa*, with markers in Linkage Groups I and VI. Conidia were irradiated with small doses of ultraviolet light prior to use in a cross. When compared with untreated crosses, two of three treated crosses showed highly significant increases in crossing-over in the region between *cr* (crisp) and *arg-3* (30300) in Linkage Group I.

Seventy-seven tetrads were analysed from treated crosses, and three were found to show atypical segregations ; in each a single locus was involved. The three loci were *hist-2* (C94), *ylo* (Y30539y), and *tryp-2* (75001). Eighty-nine tetrads isolated from similar crosses not treated with ultraviolet light, showed no atypical segregations.

## RECOMBINATION AND COMPLEMENTATION BETWEEN SUPPRESSOR GENES IN *COPRINUS LAGOPUS*

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Monocaryons of *C. lagopus* carrying a gene *pur*, which affects the synthesis of aromatic compounds and gives rise to purple pigmentation, display instability due to suppression of *pur* by recessive mutations at other genes. Suppressed cultures are thus white, although not always of wild phenotype.

When matings are made between suppressed monocaryons homozygous for *pur* but carrying suppressors of independent origin, complementation between suppressors may occur—restoring the purple phenotype. Some 40 suppressors have been tested for complementation with each other and can be arranged on a single linear complementation map. Preliminary recombination analysis on the other hand indicates that more than one suppressor locus is involved in this group. Crosses between suppressed cultures and wild type reveal recombination between *pur* and suppressor loci varying from 0 per cent. to 19 per cent. These findings support those of Lewis (1960), working with suppressors of methionine requirement, that non-complementarity may obtain between widely separated genes. It may well be significant that this phenomenon has so far only been encountered in suppressor systems. The results are of interest with respect to

(1) the concept of dominance
(2) the distinction between non-complementarity at the phenotypic level and functional identity at the genic level.

## S ALLELE MUTATIONS AND COMPONENTS OF THE S GENE

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Two components of the *S* gene complex, one controlling *S* allele specificity and having two, pollen and stylar, mutable units, and the other, determining a growth substance in the pollen, are already known. Evidence will be presented from different species of *Nicotiana* and *Solanum*, for the occurrence of (1) another growth substance unit controlling production in the style and (2) a third component, determining a general primary specificity and having pollen and stylar mutable units.

On the above basis of *S* gene structure, eight kinds of *S* allele mutants are possible, six of which have already been identified. In the light of these observations, the reason for the failure to recover spontaneous or induced mutations of the normal *S* allele in certain solanaceous species will also be briefly discussed.